The neural correlates of disgust : a multimodal investigation

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The Neural Correlates of Disgust - A Multimodal Investigation Using Functional Magnetic Resonance Imaging

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Abstract

Lesion and functional imaging studies have suggested distinct neural areas underlying the perception of different emotions independent of sensory modality of stimulus presentation. The insula has been implicated in the perception of disgust and the amygdala in the perception of fear. Other studies have investigated neural substrates underlying the perception of happiness, anger and sadness but with less clear-cut results. The majority of previous studies has employed visual stimuli.

The aim is to investigate the following questions:
(1) What are the neural correlates underlying perception of specific emotions, especially disgust?
(2) Is the neural substrate underlying perception of a specific emotion, especially disgust, independent of the sensory modality (vision, hearing, olfaction, gustation) in which the stimuli are presented?

To achieve this, I have completed an fMRI study of visual and auditory perception of disgust, fear, anger and sadness. The results have partly replicated previous findings of amygdala activation in response to fear and insula activation in response to disgust. The results also suggest an effect of the context in which an emotional stimulus is presented. This context effect was investigated in a follow-up study using visual stimuli and focusing on the perception of disgust when preceded by other emotions. It shows habituation of the insula in response to repeated exposure of disgusting facial expressions, and an abolished insula response when the presentation of disgusting stimuli is preceded by a variety of other emotive stimuli.

fMRI studies investigating the neural correlates of perception of disgusting, unpleasant but not disgusting and pleasant odours and tastes have also been completed. The results support the hypothesis that the anterior insula is involved in the perception of disgust regardless of sensory modality of stimulus presentation and forms a key component of the neural circuitry underlying this emotion.

TABLE OF CONTENTS

List of Figures

List of Tables

Chapter 1

Introduction

1.1 General approach used in this thesis

Emotions play a central role in human life, forming the basis of our social relationships as well as influencing the decisions made in the course of everyday life (Damasio, 1994). This is illustrated by the case of Phineas Gage, who in 1848 sustained frontal lobe damage when an explosion caused an iron rod to enter his cheek and exit through the top of his skull. After the accident he was first thought to have made a full recovery, as there was no speech, motor or memory impairment. It only became evident later that he had undergone a profound personality change, which left him unable to form meaningful relationships or make advantageous decisions. This is the first case, which demonstrates that there are specific brain areas not just for perception, motor function etc but also for personal and social dimensions of reasoning (Damasio, 1994). Developing an understanding of emotions can not only provide insights into the human condition, but is of great importance in the drive to develop treatments for debilitating disorders of emotion such as depression and anxiety.

In non-human animals, some progress has already been made in understanding the neural basis of emotion, mostly on the basis of investigations carried out into the structure and function of some key brain regions such as the orbitofrontal cortex, the amygdala, or the ventral striatum. The underlying assumption behind such research is that an understanding gained from the study of these brain regions in non-human animals is directly applicable and relevant to understanding the structure and function of what are presumed to be homologous regions in the human brain. At present, very little is known about the functions of these regions in the human brain however, and it is not yet clear to what extent this assumption is valid. With the recent emergence of brain imaging techniques such as PET (positron emission tomography) or fMRI (functional magnetic resonance imaging), a means of studying the functions of the human brain in vivo has become available. In concert with human lesion studies, these neuroimaging techniques provide the opportunity to investigate functions of different regions of the human brain, and determine the neural basis of human emotions.

In order to further an understanding of human emotions, the approach taken in this thesis is to study the neural basis of the response to emotive stimuli presented in different sensory modalities: visual, auditory, olfactory and gustatory, using functional magnetic resonance imaging. The emotion of particular interest is disgust because of the ability to experience disgust in response to stimuli presented in the four sensory modalities mentioned above.

Emotion perception can be understood in terms of three related processes (Phillips, 2003). Firstly, the identification of emotionally-salient information in the environment. Secondly, the production of an affective state and emotional behaviour in response to this information. And thirdly, the regulation of the affective state and emotional behaviour. The main focus of this thesis is the first stage: identification of emotionally-salient information in the environment. In the olfactory and gustatory modalities this is closely associated with the production of an affective state, and it is not always possible therefore to separate the two phenomena.

In this chapter an overview of some of the main theoretical approaches to emotion and the neurobiology of emotion, especially of disgust, will be presented, together with an outline of the anatomy of the insular cortex and the neurobiology of the sensory systems in which the stimuli are presented: visual, auditory, olfactory and gustatory systems.

1.2 Brief review of theories of emotions

One of the earliest psychological theories of emotions is a dualist, or "feeling", theory proposed by James (1910, first proposed in 1884), which describes emotions as epiphenomena, or non-functional feelings, separate from the physiological changes or behaviour in response to provoking stimuli. James proposed that
"*The bodily changes follow directly the perception of the exciting fact, and that our feeling of the same changes as they occur is the emotion*" (James, 1910).

This theory has received many criticisms throughout the years. One of the most cogent critiques was put forward by Cannon (1927). Cannon's main criticism is that total separation of the viscera from the central nervous system does not abolish emotional behaviour, which he demonstrated in cats with spinal cord lesions. Other evidence

comes from a more recent investigation of humans with spinal cord lesions, who do not show a decrease in emotions (Bermond et al., 1991). Cannon also investigated the function of physiological changes accompanying many emotions. Such peripheral reactions are of great importance in preparing particular organ systems for particular types of action and in returning such systems to a basal state when such preparedness is no longer required. One example given by Cannon is a frightened animal, which can either run away or fight. The release of adrenaline in response to a fearful stimulus would not only ready the animal for flight but also speed coagulation of blood and hence reduce blood loss, which could occur during a fight. Cannon was of the opinion that although bodily feedback was not needed to distinguish between emotions, it nevertheless played an important role, giving emotions their characteristic sense of urgency and intensity.

Behaviourist or learning theories define emotions in terms of reinforced patterns of behaviour (Gray, 1975; Rolls, 1990, 1999). The basic idea behind a theory of this sort is summarised by Gray in his definition of emotion:

> [Emotions are] ..."*those internal states of the CNS which are produced by instrumentally reinforcing events or by stimuli which have in the subject's previous experience been followed by instrumentally reinforcing events*" (Gray, 1975).

A version of this theory has also been put forward by Rolls (1990, 1999). Rolls (1999) states that emotions are "*states elicited by rewards or punishers*". As in the earlier theory of Gray, it is proposed that the particular emotion produced depends on whether the reinforcer is positive or negative and the reinforcement contingency.

Cognitive appraisal theories, dating from Aristotle, emphasise the importance of cognitions as causal to emotions, with theorists such as Lyons (1992) describing the appraisal of interpretation of events, which then leads to physiological changes, as central to the formation of an emotion. In its simplest terms, appraisal can be considered to be the assessment of a situation in terms of its significance for the organism such as the potential harm or benefit that could result (Arnold, 1960). Cognitive appraisal is clearly important in determining whether an environmental stimulus will give rise to an emotion, which emotion it will give rise to, and which behaviour actually results from that emotion. For example, the capacity of a PhD viva voce examination to induce

anxiety clearly depends on cognitive evaluative factors (McNaughton, 1989). Likewise, when an emotional state is produced the observed behaviour of an animal can depend markedly on its assessment of, for example, the status of other animals close to it.

Ekman (1992b) has described emotions as "having evolved through their adaptive value in dealing with fundamental life-tasks". Similar to Darwin (1872/1998), he argues that emotions are characterised by several unique features, including a distinctive facial expression, distinctive physiology, presence in other primates and distinctive antecedent events (Ekman, 1992b). Intact perception and experience of emotion would thus appear to be vital, in evolutionary terms, for survival in the social environment.

Another important question is how many emotions there are. One theory (Cannon, 1927; Lyons, 1992) argues against separate, basic emotions, but instead suggests that a general level of arousal will be interpreted by the individual in terms of the events and evaluations with which it is associated. Davidson (1992) has proposed a single emotion dimension built upon primitive adaptive responses: approach (positive) through to withdrawal (negative). There are limitations to this theory as there is no definite evidence to show that all positive emotions always involve approach. There is evidence showing that negative emotions such as anger, fear or disgust can involve approach or withdrawal. Rolls (1999) classified different emotions according to a two-dimensional scheme, where the ends of each axis represent the type of the reinforcer and the nature of the contingency. This theory accounts for many separate emotions that are distinctly different from each other. A similar two-dimensional scheme (Lang et al., 1990, 1993) is based on arousal on one axis and valence on the other. This also accounts for the existence of different, separate emotions. Gray (1994) has proposed three fundamental emotion systems: a behavioural approach system, a fight/flight system, and a behavioural inhibition system. Each system can be described in terms of its behavioural reinforcement-related inputs and outputs, the neural systems underlying it, and the information processing associated with it at the cognitive level. Gray stresses the need to distinguish between a fundamental emotional state caused by activity in one of these three fundamental emotion systems and the experience of different emotions, which corresponds to a blend of activity in all three systems. This theory can therefore account for the existence of a large number of emotions, but does not attempt to quantify them.

Another type of theory (Darwin, 1872/1998; Ekman, 1992b, a) argues for the existence of separate, basic emotions, proposing six: sadness, happiness, anger, surprise, fear and disgust, each characterised by a distinctive facial expression, physiology, antecedents and presence in other primates (Ekman, 1992b). Indeed, a particularly close association between systems specialized to react to emotive stimuli in different modalities, whether detected directly by the individual or indirectly by way of conspecific facial expression, is likely to have aided survival. This theory has become more popular in recent years as studies have examined the neurobiological substrates for different emotions.

1.2.1 Disgust

Disgust is believed to have evolved as an emotion to protect the individual from danger in the form of harmful substances, including those detected in the olfactory and gustatory modality. Disgust (literally, "bad taste") has been defined in terms of a food-related emotion. It has been recognised as a basic emotion since Darwin (1872/1998), who wrote that disgust was "...something offensive to the taste". Later authors describe the emotion as "revulsion at the prospect of (oral) incorporation of an offensive object" (Rozin & Fallon, 1987). Sources other than ingestion, such as sex or defence again infection (Rozin et al., 2000) have been proposed. It has also been argued that disgust is based on the senses of touch and smell, and that taste has become associated with disgust more recently (Miller, 1997). Nevertheless, it is broadly accepted that disgust does have a food origin due to its characteristic facial expression, which can be seen as rejecting food, and having nausea as a physiological concomitant, which inhibits oral intake.

Rozin and Fallon (1987) introduce the concept of core disgust, which requires three components: oral incorporation, offensiveness, and contamination potency. Core disgust has its origin in food rejection and can be differentiated from three other categories of food rejection. These categories are distaste, in which food is rejected due to its negative sensory properties, danger, which is food rejection based on potential harm to oneself, and inappropriateness, rejecting food which is inedible such as paper. Disgust differs from the above because in this case the basis for food rejection is ideational rather than practical. A food item can be edible, harmless, tasty, and yet perceived as disgusting,

such as chocolate in the shape of dog excrement, or a drink which briefly had a sterilised dead insect in it (Rozin & Fallon, 1987).

This concept of core disgust can be expanded to include body products, animals, sexual behaviour, contact with death and dead bodies, poor hygiene and violation of body borders at points other than the mouth (Rozin & Fallon, 1987, 1994). The concept of disgust can be even further elaborated to include interpersonal contamination, with disgust elicited by physical contact with unpleasant or unknown people (Rozin et al., 1994); and, finally, the moral or socio-cultural domain of the emotion with disgust at certain beliefs or behaviours, such as sexual abuse of children, acting as a powerful means of transmitting social values (Rozin & Fallon, 1987). It has also been argued that other, complex emotions, such as shame, guilt and embarrassment, are derived form the basic emotion of disgust, with the focus of disgust on the self (Power & Dalgleish, 1997).

Like other emotions, disgust has a characteristic facial expression (Ekman & Friesen, 1976), which involves facial muscles necessary for the avoidance of ingestion of contaminants (Rozin et al., 1994) and consists of closing of the nostrils and opening of the mouth. The purpose of the facial expression is to inhibit ingestion of the repulsive object, the nostrils closing off serves to reduce the input of an offensive odour, whereas the opening of the mouth allows expulsion of offensive objects. The facial expression is usually accompanied by nausea, a physiological manifestation of rejection by reverse peristaltis and disgust. This is often associated with the consequences of ingestion of harmful stimuli and serves to discourage further or future ingestion. According to Darwin (1872/1998) the physiological concomitants of disgust are the phylogenetic remainders of a voluntary vomiting system.

It is thought that disgust in its wider meaning after the core disgust is absent at birth and develops through childhood (Rozin & Fallon, 1987), since very young children do not display an aversion to faeces or decaying materials and display hyperorality. In fact, it seems that the only category of food rejection present is distaste, as the characteristic facial expression of disgust including the opening of the mouth can be observed both in human infants and in rats presented with a bitter stimulus (Rozin & Fallon, 1987). It

seems that in the first years of life children learn what not to eat and learn about disgust. The emotion of disgust therefore appears to be closely related to distaste.

The output system of disgust including the characteristic facial expression appears to have remained stable during cultural evolution, and the main difference during development and across cultures appears to be the input system, the stimuli that elicit disgust (Rozin et al., 2000). As disgust appears to have evolved to enable the avoidance of ingestion of harmful substances, recognition of disgust-provoking stimuli, presented in the olfactory and gustatory modality in particular, is important, in addition to the recognition of facial expressions of disgust in others to facilitate vicarious learning of the avoidance of these substances. A neural system specialised for the identification of and response to disgusting stimuli presented in different sensory modalities would therefore be plausible.

1.3 The neurobiology of emotions

At present there is limited understanding of the neurobiological bases of the different processes underlying emotion perception. In the past two basic systems underlying different forms of emotion have been proposed: one mediating approach, the other mediating withdrawal (Cacioppo & Gardner, 1999; Davidson & Irwin, 1999; Gray, 1994). A third system mediating behavioural inhibition has been proposed by Gray (1994). In this chapter, instead of focussing on an approach-withdrawal division of the neuroanatomy underlying emotion, I am going to discuss the potential neural bases of three processes important for all emotion perception (Phillips, 2003): 1) the identification of emotionally-salient information in the environment; 2) the production of an affective state and emotional behaviour in response to 1), which, in turn, may bias 1) towards the identification of specific categories of emotional stimuli; and 3) the regulation of the affective state and emotional behaviour. This may involve an inhibition of processes 1) and 2), so that the affective state and behaviour generated in response to environmental stimuli are contextually appropriate. Critical to survival is the ability to identify quickly in the environment emotionally-salient information, including danger and reward, and to form rapid and appropriate behavioural responses (Darwin, 1872/1998). The presence of an emotion generally involves physiological arousal, appraisal, subjective experience, expression, and goal-directed behaviour (Ekman,

1992b), but there is still some debate about the neurobiological bases of the different processes underlying emotion perception. The evidence for the neural substrates underlying emotion perception is based on findings from recent animal, human neuropathological and functional neuroimaging studies.

The "limbic" circuit, first proposed by Papez in 1937, comprised of rostral/ pregenual anterior cingulate gyrus, ventromedial prefrontal (orbitofrontal) cortex, ventral striatum (including the nucleus accumbens and medial olfactory tubercle), ventral pallidum, substantia nigra, and dorsomedial nucleus of the thalamus, has been identified as potentially important for motivation and emotion processing (Alexander et al., 1990). Other important connections include those from the amygdala to the anterior cingulate gyrus and ventromedial prefrontal cortex (Alexander et al., 1990), and those from the anterior (agranular) insula to the amygdala, ventromedial prefrontal cortex and rostral anterior cingulate gyrus (Augustine, 1996). The retrosplenial cortex is also thought to be involved in emotion processing, as has been proposed by a recent review of functional neuroimaging studies of emotion (Maddock, 1999).

In the following chapter I will discuss the evidence for the roles of the ventral striatum, the amygdala and anterior insula in the identification of emotionally-salient information. In section 1.3.2 I will examine the evidence for the roles of these structures, together with medial prefrontal cortical regions, in the generation of emotional states and behaviours. In section 1.3.3, I will then discuss the evidence for the roles of the hippocampus and prefrontal regions in the regulation of emotional behaviour.

1.3.1 The identification of emotionally-salient information in the environment

Findings from studies employing a variety of different techniques have highlighted the importance of the ventral striatum in the response to emotionally-salient information. The existence of brain regions specialized for reward processing was initially suggested by studies showing that rats responded operantly to stimulation of specific sites to the exclusion of other activities (Olds & Milner, 1954). These included the midbrain dopaminergic projections from the ventral tegmental area into the nucleus accumbens shell region and the medial prefrontal cortex (Spanagel & Weiss, 1999). However, in rodents dopamine release in the nucleus accumbens can also be caused by aversive

stimuli (Levita et al., 2002). In primates, the nucleus accumbens, the ventral putamen and the medial portion of the caudate head are involved in reward processing, and they receive projections from the amygdala and the orbital and medial PFC (Ongur & Price, 2000; Rolls, 1999). In human functional neuroimaging studies employing PET, the euphoric response to dextroamphetamine correlates positively with the magnitude of dopamine release in these anteroventral striatal areas. This dopamine signal may be important for the formation of associations between salient contextual stimuli and internal rewarding events (Drevets et al., 2001). Infusion of cocaine has been shown to cause activation in the ventral striatum in cocaine addicts, just as nicotine causes activation in the nucleus accumbens in smokers (Davidson & Irwin, 1999). Another study (Sutton, 1997) reports activation in the nucleus accumbens during picture-induced positive affect. These studies support a role for the ventral striatum in the processing of reward.

The amygdala is a small, almond-shaped region within the anterior part of the temporal lobe. It is located close to nuclei within the striatum, especially the nucleus accumbens, and also the putamen and caudate nucleus. Cells in the amygdala which respond selectively to faces and the direction of eye gaze have been identified in studies of non-human primates (Brothers & Ring, 1993) and humans (Heit et al., 1988). Studies of patients with amygdala lesions have highlighted the role of the amygdala in facial recognition and gaze direction perception (Young et al., 1995), in addition to emotional expression, particularly threat and fear, identification in visual (Adolphs et al., 1994) and auditory (Scott et al., 1997) modalities of presentation.

Studies employing functional neuroimaging techniques have demonstrated increased blood flow and activation within the amygdala in response to unfamiliar faces (Dubois et al., 1999), and to presentations of fearful (Breiter et al., 1996; Morris et al., 1996), sad (Blair et al., 1999; Schneider et al., 1997), and happy facial expressions (Morris et al., 1996). Other studies have demonstrated activity within the amygdala to emotive scenes (Lane et al., 1997b; Lang et al., 1998; Taylor et al., 2000), film excerpts (Reiman et al., 1997), and to presentations of fearful and angry facial expressions of which the observer had no conscious awareness (Whalen et al., 1998). However, Phillips et al. (2004) did only find amygdala activation in response to overt presentation of fearful faces, but not in response to covert presentations. The amygdala has been implicated in the response

to visual presentations of threatening words (Isenberg et al., 1999), fearful vocalisations (Phillips et al., 1998b), and to unpleasant olfactory (Zald & Pardo, 1997), and gustatory (O'Doherty et al., 2001b; Zald et al., 1998) stimuli, in the memory for emotional information (Dolan et al., 2000; Hamann et al., 1999), and in the enhanced perception of emotionally-salient information (Anderson & Phelps, 2001).

These findings suggest a specific role for the amygdala in the perception of emotionally-salient information per se and in the modulation of attention to emotional stimuli. It has been suggested that this modulation may occur via projections from the central nucleus of the amygdala to cholinergic neurons which lower cortical neuronal activation thresholds and potentiate cortical information processing (Whalen, 1998), for example, within the visual cortex (Morris et al., 1998).

The insula is a part of the cerebral cortex at the base of the lateral fissure. Its anatomy, connectivity and functions are described in more detail in section 1.3.4. Here, I will only give a brief overview. In experimental animals, lesions which include the anterior insula and adjacent ventrolateral PFC reduce fear reactivity to contextual stimuli, but do not affect CS acquisition or response extinction (Morgan & LeDoux, 1999). In humans, functional neural imaging studies have highlighted the role of the insula in delay fear conditioning (Buchel et al., 1999), and during the anticipation of an aversive stimulus (Phelps, 2001), suggestive of a role for this structure in conveying the representation of aversive, sensory information to the amygdala.

The insula, together with the ventral striatum and thalamus have also been implicated in the identification of displays of another emotion, disgust. A recent examination of a patient with a focal insula lesion has highlighted the importance of the insula in the recognition of facial and vocal expressions of disgust in humans (Calder et al., 2000). Patients with Huntington's Disease, in whom there is degeneration of the caudate nucleus, also demonstrate an impairment in the recognition of facial expressions of disgust (Sprengelmeyer et al., 1996). Functional neuroimaging studies have provided evidence for the role of the insula in the identification of facial expressions of disgust (Phillips et al., 1997; Sprengelmeyer et al., 1998), in taste perception (Small et al., 1999) and in olfaction (Royet et al., 1999, 2001; Savic et al., 2000; Zald & Pardo, 2000b).

These findings indicate roles for the amygdala and insula in the identification of the presence of emotionally-salient information, but also suggest a predominant role for the amygdala in the perception of negative emotional material, ambiguity and threat, and a role for the insula in the identification of disgust (Calder et al., 2001).

1.3.2 Entering into an affective state and emotional behaviours

In a recent model, the importance of the internal representation of bodily states during the generation of emotion has been emphasised (Damasio, 1999). First-order mapping of bodily states includes the mapping of homeostatic autoregulatory processes, and involves the midbrain and upper pons, the hypothalamus and thalamus, somatosensory cortex and insula (Damasio et al., 2000), whilst second-order mapping includes the mapping of experience-dependent changes, and involves anterior and posterior cingulate gyri (Critchley et al., 2001). This suggests that these regions are important for the generation of emotional states. In this chapter, I will focus upon the specific roles of the following regions: the amygdala, the insula and ventral regions of the anterior cingulate gyrus and prefrontal cortex.

Bilateral lesions of the temporal lobes result in significant changes in social behaviour in monkeys (Kolb, 1996), including hyperorality, social disinhibition and an absence of emotional motor and vocal reactions usually associated with stimuli eliciting emotional states. This has been termed the Klüver-Bucy syndrome, after the scientists who first described it. Initially it was thought that this was especially due to amygdala damage, but it has since been shown that discrete amygdala lesions produce nothing resembling the Klüver-Bucy syndrome (Kalin et al., 2001). In fact, neonatal amygdala lesions in monkeys produce a decrease in fear of objects that monkeys would not normally approach, such as snakes, but an increase in social fear (Prather et al., 2001). Although the Klüver-Bucy syndrome has also been observed in humans (Kolb, 1996), distinct amygdala lesions in humans result in deficient nonverbal visual memory and social behaviour (Tranel & Hyman, 1990), reduced fear conditioning and a lack of a startle potentiation in response to aversive visual stimuli (Davidson, 2002). Studies employing functional neuroimaging techniques have demonstrated amygdala activation in response to induction of positive and negative emotional states (Reiman et al., 1997; Schneider et

al., 1997), and during fear conditioning paradigms (Buechel et al., 1998, 1999; LaBar et al., 1998). These findings suggest that the amygdala might be necessary for the expression of negative affect.

Functional neuroimaging studies have also highlighted the role of the insula during recall of internally-generated emotion (Reiman et al., 1997), and during the experience of guilt (Shin et al., 2000), a complex emotion which, like the experience of shame, may involve self-directed disgust (Power & Dalgleish, 1997). There is therefore accumulating evidence for the role of the insula in mediating behaviour to aversive, including disgust-related, stimuli. For a more detailed description of the structure and function of the insular cortex see section 1.3.4.

It is the ventral division of the anterior cingulate gyrus which has most consistently been associated with emotional behaviour. This region has extensive connections with the amygdala, periaqueductal grey, mediodorsal and anterior thalamic nuclei, nucleus accumbens and ventral striatum, and has been associated with autonomic function and emotional behaviour (Devinsky et al., 1995). Lesions of this region in the rat result in a significant impairment of the ability of the autonomic system to respond to conditioned stimuli (Cardinal et al., 2002). In humans, functional neuroimaging studies have implicated the ventral part of the anterior cingulate in both normal sadness and in depression (Liotti et al., 2000; Mayberg et al., 1999), as well as in the perception of emotionally significant stimuli (Cardinal et al., 2002). Studies of healthy volunteers have demonstrated increased blood flow and activation within the anterior cingulate gyrus during mood induction compared with a resting state (George et al., 1995; Mayberg et al., 1999; Schneider et al., 1997; Shin et al., 2000; Teasdale et al., 1999). The ventral anterior cingulate gyrus therefore appears to have an important role in the evaluation of emotional information during arousal and the generation of emotional states.

There is evidence from a range of studies employing different techniques for the role of the ventromedial prefrontal cortex, and the orbitofrontal cortex in particular, in the generation of emotional states and behaviour (Davidson, 2002). Orbital and ventromedial prefrontal cortex is situated medially on the ventral surface of the prefrontal cortex. It has direct connections from the basolateral nucleus of the amygdala,

and appears to have a critical role in the representation of the reward value of a stimulus and the way in which this representation then guides goal-directed or choice behaviour (Rolls, 1999).

Lesions to the orbitofrontal cortex in monkeys produce emotional changes, including reduced aggression to humans, changes in food-selection behaviour, and an impaired ability to break a learned association between a stimulus and a reward when this becomes appropriate (Rolls, 2000). Orbitofrontal cortical neurons in monkeys have been demonstrated to respond during taste and smell (Rolls, 2000; Rolls & Baylis, 1994; Rolls et al., 1996) perception, but also to visual and oral somatosensory stimuli associated with reward (Rolls, 2000).

In humans, lesions to the orbitofrontal cortex result in impaired emotional expression identification (Harmer et al., 2001; Hornak et al., 1996), but also lead to disinhibition, impulsiveness and misinterpretation of other people's moods (Damasio, 1994). Lesions of the orbitofrontal cortex can also result in the failure to alter behaviour when stimulus-reinforcement associations change (Damasio, 1994; Rolls, 2000). Lesions of ventromedial prefrontal cortex are associated with impaired performance on gambling tasks designed to measure decision-making regarding high- versus low-risk options for monetary reward (Bechara et al., 1999, 2000). Single-neuron responses have been recorded in human ventral prefrontal cortex to aversive visual stimuli (Kawasaki et al., 2001).

Functional neuroimaging studies have demonstrated increased blood flow and activation within the orbitofrontal cortex during the perception of pleasant and unpleasant odours, flavours and tactile stimuli (Francis et al., 1999; O'Doherty et al., 2001b; Zald et al., 1998; Zald & Pardo, 1997). Other functional neuroimaging studies have demonstrated increased activation of the orbitofrontal/ventromedial cortex during the perception of odours of foods not eaten to satiety compared with those of foods eaten to satiety (O'Doherty et al., 2000), during the performance of gambling tasks (O'Doherty et al., 2001a), and during guessing and decision-making on the basis of reward value (Elliott et al., 2000). Finally, increased regional cerebral blood flow within the orbitofrontal cortex has also been demonstrated during imagined anger (Dougherty et al., 1999; Kimbrell et al., 1999), and during imagined restraint of physical aggression compared

with imagined aggressive behaviour (Pietrini et al., 2000), although decreased cerebral blood flow to this region has been demonstrated during imagined physical aggression compared with neutral behaviour (Pietrini et al., 2000). Taken together, these findings suggest a role for the orbitofrontal and ventromedial prefrontal cortex in representing primary reinforcers, learning associations of other stimuli with these reinforcers, and decision-making about behavioural responses to emotionally-salient material, especially when the reinforcement contingencies change. The findings from the above studies indicate that ventral regions of the anterior cingulate gyrus and ventromedial regions of the prefrontal cortex are important for the production or recognition of autonomic changes associated with affect states and emotional behaviours.

The ventrolateral prefrontal cortex can be defined as lateral and rostral regions of Brodmann area 47 and part of Brodmann area 45, and lies lateral to the orbitofrontal cortex on the ventral surface of the frontal lobes. Its role in the generation of emotional states and behaviour is less clear. Human functional neuroimaging studies have demonstrated increased blood flow and activation within this region during a variety of tasks, including the induction of sad mood (Pardo et al., 1993) and guilt (Shin et al., 2000), during the recall of emotional material (Reiman et al., 1997), and in response to facial expressions displaying different negative emotions (Sprengelmeyer et al., 1998). The right temporofrontal junction and related right-sided ventrolateral prefrontal cortex have also been associated with autobiographical memory retrieval (Markowitsch, 1997). These findings suggest a role for the ventrolateral prefrontal cortex in the generation of emotionally-salient associations, which may involve emotional and autobiographical information.

1.3.3 The regulation of emotional behaviour

As this thesis focuses on the perception of emotions and not on the modulation or regulation of the emotion or the emotive behaviour I am going to only briefly outline the brain areas involved in the regulation of emotional behaviour.

The regulation of emotional behaviour allows the behaviour to be appropriate in the context in which it occurs. Many of the regions important for the generation of affect states and emotional behaviour, including ventromedial and ventrolateral prefrontal

cortices, are important for emotional behavioural regulation involving an unconscious, and automatic response to emotional stimuli. In this section, I describe the evidence for the roles of other prefrontal cortical regions and of the hippocampus in the regulation of emotional states and behaviour, including those regions associated with the performance of non-emotional, cognitive tasks, and those important for the attention to and manipulation of emotional information. These include the cognitive division of the anterior cingulate gyrus, which is dorsal to the affective division, and the dorsomedial and dorsolateral prefrontal cortices (Devinsky et al., 1995; Drevets, 2000).

The predominant role of the dorsal, cognitive, division of the anterior cingulate gyrus appears to involve the regulation of attention and motor behaviour. In humans, lesions of this region are associated with visual and somatosthetic neglect, attentional deficits and impaired performance on tasks requiring controlled processing (Devinsky et al., 1995). Electrical stimulation of this region in humans is associated with a range of behavioural changes, including primitive movements and automatisms similar to those reported in patients with complex partial seizures (Devinsky et al., 1995). Several studies employing functional neuroimaging techniques have demonstrated increased blood flow within this region during performance of tasks requiring selective attention and response selection when novel choices are required (Cardinal et al., 2002).

Increasing evidence, however, indicates a role of the dorsal anterior cingulate gyrus in processing emotionally-salient information. Increased regional cerebral blood flow to rostral and dorsal regions of the anterior cingulate gyrus has also been reported during attention to subjective emotional states and experiences (Lane et al., 1997a, 1998). Studies employing functional neuroimaging techniques have demonstrated that dorsal regions of the anterior cingulate gyrus are important for the encoding of the perceived unpleasantness of pain (Davis et al., 1997; Peyron et al., 2000; Schnitzler & Ploner, 2000; Treede et al., 1999). Finally, dorsal regions of the anterior cingulate gyrus are activated during the regulation and second-order mapping of internal bodily states (Critchley et al., 2001). These findings indicate that the dorsal anterior cingulate gyrus is important for the direction of attention to and the representation of information about internal and emotional states, and may therefore have a predominantly executive or regulatory role in emotion processing, rather than facilitating the generation of emotional states.

Findings from a range of different studies of animals and those employing functional neuroimaging techniques in humans have indicated that dorsomedial and dorsal anterolateral prefrontal cortices are important for the modulation of emotional responses (Drevets, 2000). These regions border the dorsal anterior cingulate gyrus and lie medial to the dorsolateral prefrontal cortex. The dorsolateral prefrontal cortex, which in humans includes Brodmann areas 44 and 46, has been demonstrated to be important for the performance of tasks of working memory, the maintenance of information in short-term memory. Some studies have also shown increased blood flow and activation within this region in response to positive and negative facial expressions during the performance of explicit, emotion labelling tasks compared with more implicit tasks (Hariri et al., 2000; Nakamura et al., 1999). The dorsolateral prefrontal cortex may therefore be associated with the representation and manipulation of the non-emotive visuospatial and verbal components of emotional stimuli, rather than the identification of emotive information per se.

The hippocampus also plays a role in the regulation of emotional behaviour. The hippocampus has been implicated in the inhibition of the stress response via inhibitory connections with many of the structures involved and activated in the stress response, including the amygdala, the paraventricular nucleus of the hypothalamus, and the locus coeruleus (Phillips, 2003). The hippocampus has also been proposed as a comparator monitoring whether the current state matches the expected state, underlying the behavioural inhibition system (Gray, 1982). This has been expanded in the Gray-McNaughton theory (Gray & McNaughton, 2000), which proposes that the hippocampal formation responds to aversive stimuli whenever they occur during a behavioural conflict. It resolves this conflict by amplifying the neural representation of the aversive stimulus, thereby biasing attention to this stimulus and preparing the organism for the worst possible outcome. This theory has been supported by an event-related fMRI study demonstrating a role for the hippocampus in anxiety influences on responses to pain (Ploghaus et al., 2001). When participants were more anxious, physically identical noxious stimuli were perceived as more painful. This anxiety induced hyperalgesia was associated with increased brain activation in the hippocampal area. It suggests that during anxiety the hippocampal formation sends signals amplifying the neural representation of the painful stimuli (Ploghaus et al., 2001, 2003). Based on the animal literature and on human morphometric studies it has also been proposed that the

hippocampus plays a role in the context-regulation of emotional behaviour in humans (Davidson et al., 2000).

In this section, I have described findings from studies investigating the neural basis of the neuropsychological processes important for emotion perception, namely: the identification of emotionally-salient information in the environment; the production of an affective state and emotional behaviour in response to this; and the regulation of the affective state and emotional behaviour. These processes appear to be dependent upon the functioning of two parallel neural systems: 1. a ventral, or "limbic", system important for the identification of emotional information and the generation of affect states; and 2. a dorsal system, important for the performance of executive functions, including selective attention, planning, and motor responses to emotional stimuli, and including regions where cognitive processes are integrated with and can be biased by emotional input. There appears to be a reciprocal functional relationship between these two systems. The extent to which a stimulus is identified as emotive, and is associated with the production of an affective state and/or emotional behaviour, may be dependent upon the relative level of activity within ventral and dorsal neural systems.

1.3.4 Functional Anatomy of the Insular Cortex

As the focus of this thesis is on the perception of disgust in different sensory modalities and the insular cortex has been shown to be especially important in the perception of disgust (Calder et al., 2001), I will describe the anatomy, connectivity and function of the insula in this section.

1.3.4.1 Structure

The insula is part of the cerebral cortex and is situated at the base of the lateral sulcus (figure 1.3.4.1a&b). It is covered by the frontal, temporal and parietal opercula. The insular cortex is a heterogeneous area, which can be divided into three cytoarchitecturally distinct divisions in the monkey arranged in the rostro-caudal direction, with increasing differentiation more caudally: an anteroventral agranular periallocortical region, a posterodorsal granular region, and a middle transitional dysgranular region (Augustine, 1996). The cellular organisation of the human insular

cortex is almost identical to that of the rhesus monkey (Flynn, 1999; Mesulam & Mufson, 1982a). As the lateral orbital frontal cortex and temporal pole show similar cytoarchitecture it has been suggested that they form one unit, a paralimbic belt, with the insula (Mesulam & Mufson, 1982a).

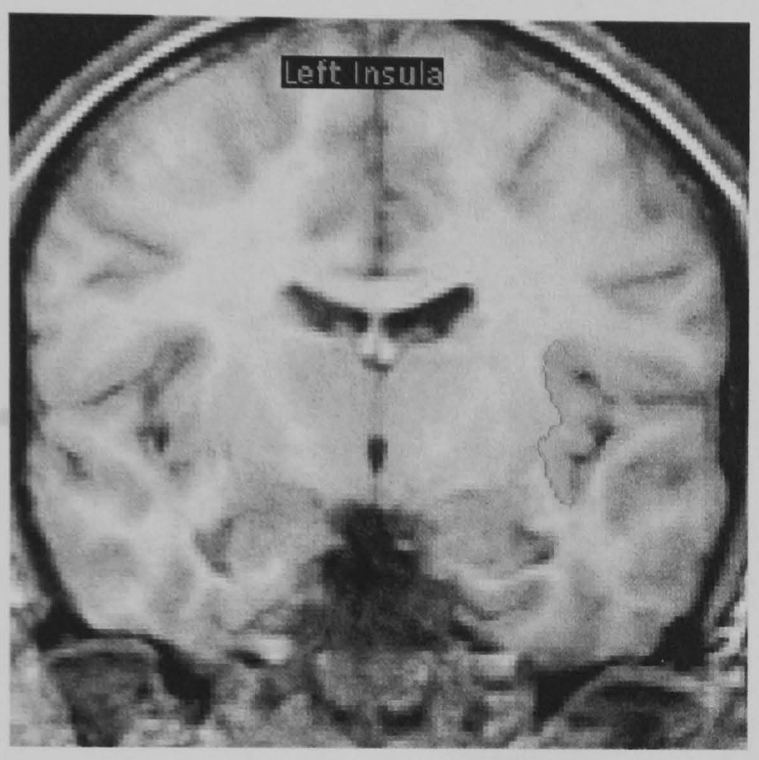


Figure 1.3.4.1a: Location of the insular cortex in the human brain
This figure shows a coronal slice of a magnetic resonance imaging scan of the human brain. Outlined in green is the insular cortex in the left hemisphere. The y coordinate of this slice in Talairach & Tournoux space is approximately -4mm (Talairach & Tournoux, 1988) (http://neuro-www.mgh.harvard.edu/cma/seg/).

The insular lobe in primates including humans has a wealth of connections with cortical areas, basal nuclei, limbic areas, amygdala and the dorsal thalamus (figure 1.3.4.1c). The insula constitutes a polymodal convergence area, whose anterior part has main connections with orbitofrontal, temporopolar and olfactory cortices, anterior cingulate and hippocampal gyri, thalamus and amygdala, and participates in olfactory, gustatory, autonomic and limbic functions. The posterior insula has main connections with the frontal, temporal and parietal cortices and the thalamus, and its functions are mainly related to somatosensory processes, auditory and skeletomotor behaviour (Mesulam & Mufson, 1982b; Mufson & Mesulam, 1982).

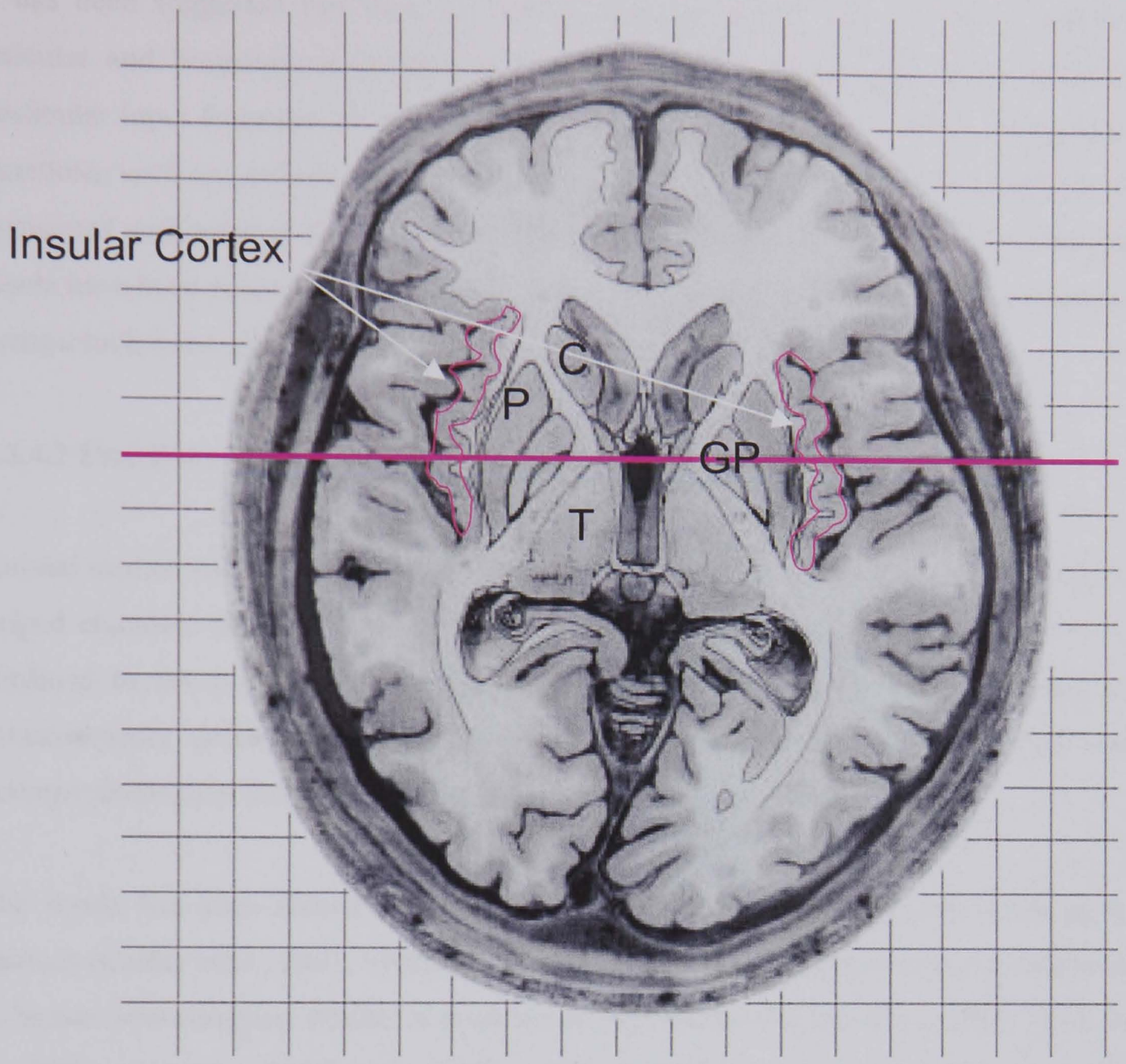


Figure 1.3.4.1b: The location of the insular cortex
This figure shows a horizontal section of a human brain. The z coordinate of this slice in Talairach & Tournoux space is approximately +1mm (Talairach & Tournoux, 1988). The insular cortex is outlined in pink. Structures of the striatum are also included in this section. Adapted from 'Atlas of the Human Brain', J.K. Mai, J. Assheuer, G. Paxinos, 1997, Academic Press, San Diego.
P = Putamen
T = Thalamus
C = Head of caudate
GP = Globus pallidus

Both anterior and posterior insula have reciprocal connections with the basal ganglia. The agranular anterior insula has projections to the ventral striatum, which form part of a limbic circuit involved in integrating feeding behaviour with reward and memory (Flynn, 1999). The posterior, granular part of the insula has connections with the dorsolateral striatum, this connection is thought to be involved in sensorimotor integration (Flynn, 1999).

It has been suggested that there are direct connections between the insula and the reticular and brainstem autonomic nuclei, in addition the posterior insula receives vestibular input from the vestibular nuclei via the thalamus (Flynn, 1999). Autonomic functions, such as cardiovascular, respiratory or blood pressure changes have also been associated with anterior insula function (Flynn, 1999), whereas the middle and posterior insula have been associated with vestibular perception, including visceral experiences of vertigo such as nausea (Augustine, 1996).

1.3.4.2 Functions

Animal studies and human neuropathological and functional neuroimaging studies have helped elucidate the functional role of the insular cortex. Stroke patients with a lesion confined to the posterior insula can present with a variety of symptoms, such as somatosensory deficits, gustatory disorders, dizziness, cardiovascular disorders, and neuropsychological deficits including aphasia (Cereda et al., 2002).

The insula has been shown to be involved in odour and taste aversion learning in animals (Calder et al., 2001; Flynn, 1999). Insula activation has also been demonstrated in human neuroimaging studies in response to both odours (Royet et al., 2000; Savic et al., 2000) and tastes (Small et al., 1997a, 1999). In human stimulation studies the insula has been shown to modulate behaviour such as swallowing, salivation, oesophagal contraction and vomiting (Penfield & Rasmussen, 1950). Patients with insula tumours have reported symptoms such as nausea, retching and vomiting, or alternating diarrhoea and constipation (Flynn, 1999). Functional neuroimaging studies in humans have demonstrated activation of the insula in response to visceral stimulation such as oesophageal distention (Aziz et al., 2000).

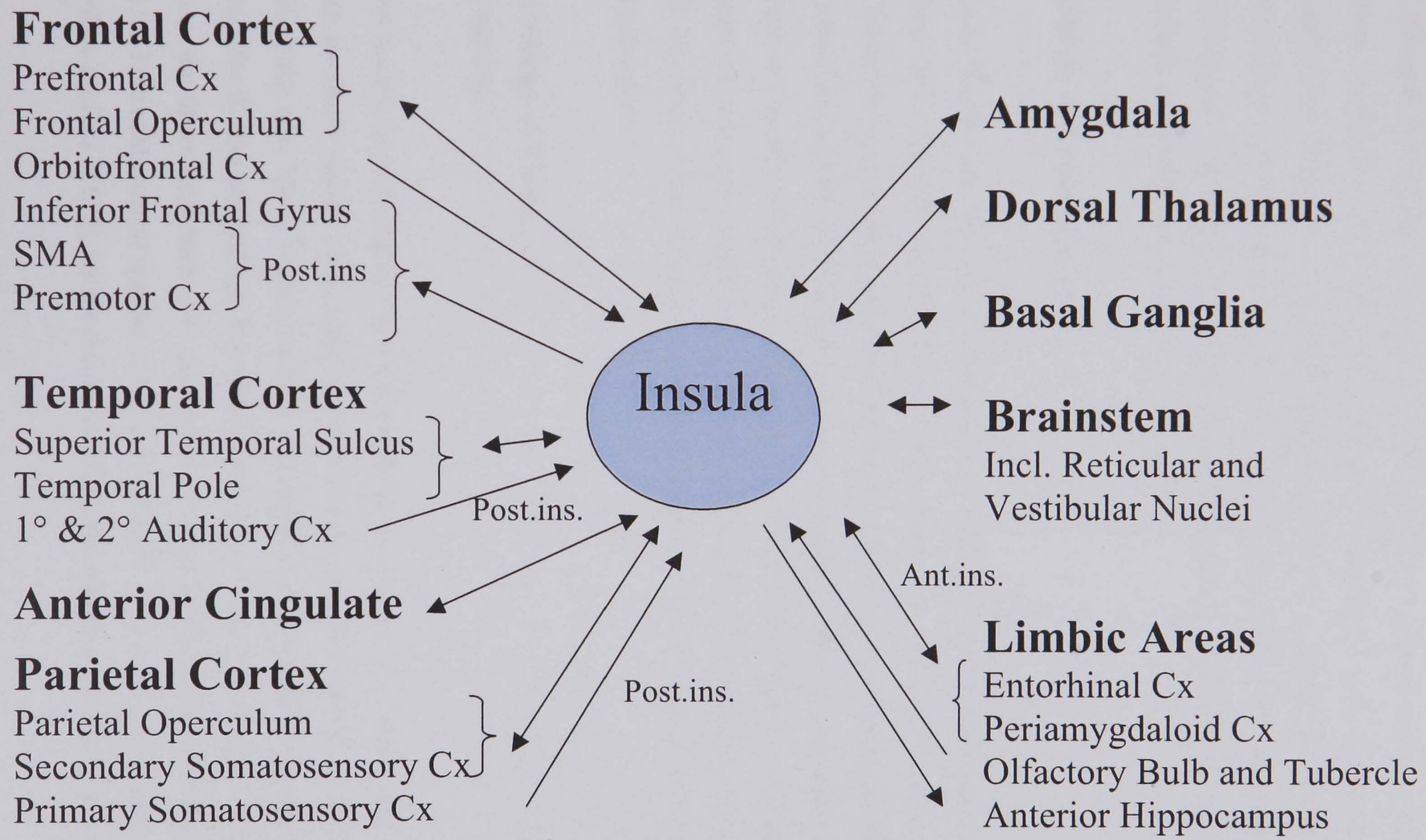
The anterior insula is also thought to be part of a nociception network, consisting of the thalamus, primary and secondary somatosensory cortices, the insula, and the anterior cingulate gyrus (Schnitzler & Ploner, 2000). Nociceptive fields have been revealed in the insula by single cell recordings and local field potentials in rats and monkeys (Schnitzler & Ploner, 2000). Lesions to the insula cause a reduction in the affective component of pain and in appropriate responses to painful stimuli (Berthier et al., 1988), and the anterior insula has been consistently activated in response to painful stimuli in

human neuroimaging studies (Peyron et al., 2000). Results of these studies suggest an integrative role of the insula, combining information about the nature of the painful stimulus from secondary somatosensory cortex and thalamus with contextual information from other sensory modalities, modifying the response, especially autonomic, to noxious stimuli, and being involved in the affective component of pain, facilitating pain-related learning and memory. The close association of the posterior insula with somatosensory function has been supported by a recent neuroimaging study showing activation of the posterior insula in response to tactile stimuli (McGlone et al., 2002). Middle and posterior insula activation has also been associated with temperature perception and was shown to correlate with perceived thermal intensity (Craig et al., 2000).

The insula also receives input from visual association and auditory cortices (Mufson & Mesulam, 1982) and has been shown to be activated in response to both auditory (Blood & Zatorre, 2001; Mirz et al., 2000) and visual stimuli (Phillips et al., 1997), and patients with insula lesions have shown a reduced emotional response to threatening visual and auditory stimuli (Berthier et al., 1988). Activity within the insula can be modulated by both awareness of visual stimuli (Critchley et al., 2002) and autonomic arousal, as patients with peripheral autonomic denervation do not show insula activation in response to threatening stimuli (Critchley et al., 2001, 2002). Furthermore, the insula has been implicated in crossmodal integration in human neuroimaging studies (Calvert, 2001).

As the insular cortex receives input from several autonomic regions and sends efferents to brain regions that play a role in regulating autonomic responses it has been suggested that the insula is critical for visceral representation (Cechetto & Saper, 1990). It has been suggested that activation of the insular cortex during emotion is associated with the autonomic changes that occur in response to an emotion (Damasio, 1999; Davidson & Irwin, 1999).

Figure 1.3.4.1c: Cortical and subcortical connections of the human insular cortex
Arrows pointing away from the insula indicate efferent connections, arrows pointing towards the insula indicate afferent connections, and double-headed arrows indicate reciprocal connections. Post.ins. = Posterior insula, Ant.ins. = Anterior insula, Cx = Cortex



1.3.4.3 Summary

In summary it can be said that the anterior insula is more involved in olfactory, gustatory, autonomic and visceral processing while the posterior insula is more concerned with integrating sensory, including somesthetic and vestibular, information and acting as a supplementary motor area (Mesulam & Mufson, 1982b). Most of the insula, particularly the anteroventral portions, have extensive connection to limbic structures. It is suggested that all the different inputs from both internal and external stimuli are integrated to allow an appropriate emotional, autonomic and behavioural response.

1.4 The Neurobiology of Sensory Perception

This chapter is concerned with the sensory modalities of vision, audition, olfaction and gustation. In this thesis disgust perception was investigated in these four sensory modalities, and therefore some background information is provided on the peripheral and central pathways involved and on relevant functional neuroimaging studies of perception in those senses. There is a wealth of functional neuroimaging studies performed in the visual modality compared with the other modalities. As the visual stimuli used in this thesis are photos of facial expressions the focus is on face perception and the neural networks involved in face perception.

1.4.1 The Neurobiology of Vision

1.4.1.1 Visual Pathways

Visual perception occurs in two stages. Phototransduction, the process by which light is converted into an electrical signal, takes place in the rod and cone photoreceptors in the retina at the back of the eye. These signals are then sent through the optic nerve to higher centres in the brain for further processing. The optic nerve fibres from each eye join at the optic chiasm where the fibres from both eyes combine according to which visual hemifield they correspond to. Three subcortical regions receive direct input from these retinal fibres: the lateral geniculate nucleus (LGN), which processes visual information, the pretectal area

of the midbrain, which is responsible for pupillary reflexes, and the superior colliculus, which controls eye movements. Here, the visual pathways will be outlined.

The LGN consists of six layers, two magnocellular ones and 4 parvocellular ones. From here, the visual information is divided into three distinct pathways that process motion, colour, and depth and form. From the LGN all three pathways project to the primary visual cortex, V1 (BA 17), and then onto secondary visual cortex, V2 and V3 (BA 18). Both in V1 and in V2 and V3 the different pathways project to different layers, which ensures that they remain separate. V4 and V5 (BA 19) are the visual association areas. V5 is located in the middle temporal (MT) area in the superior temporal sulcus and is part of the magnocellular pathway that is specialised for motion and spatial relationships. The middle temporal and middle superior temporal areas are mainly concerned with motion processing. Single-cell recordings from primate brains found motion-selective neurons in these areas. From MT the neurons project to other areas in the parietal cortex concerned with visuospatial processing. The colour pathway and the form/shape pathway on the other hand project via V1 and V2 to V4, and from there to the inferior temporal cortex. All three pathways work in parallel, with extensive interactions between the pathways, especially in the later stages of visual processing.

The human visual system is capable of recognizing an almost infinite variety of shapes. Some evidence for this pathway comes from patients with bilateral lesions on the inferior surface of the occipital lobes and the inner surface of the temporal lobes. These patients present with a syndrome called prosopagnosia, which is the impaired recognition of familiar faces. These patients can still identify parts of the face and even emotional expressions but are unable to recognize the identity of faces, sometimes they cannot even recognize themselves in the mirror. This suggests that there is a special face processing system. Indeed, some cells in the inferior temporal cortex of monkeys have been found to be responsive selectively to faces (Kandel et al., 1993).

1.4.1.2 Neuroimaging of Visual Face Processing

In this section I am going to outline the system underlying face processing with a focus on recent neuroimaging studies of face perception. Face perception is a highly developed visual skill in humans and is critical for social interactions. It is generally accepted that face perception can be divided into two distinct components: perception of identity and perception of facial expression (Bruce & Young, 1986; Haxby et al., 2002). Identity recognition is reliant on perception of fixed facial features, such as the size and shape of the nose, or the presence or absence of a dimple in the chin. Perception of facial expressions on the other hand is based on processing the changeable aspects of a face, such as eye gaze or mouth movements. These two systems need to be independent of each other, otherwise a change in facial expression could be misinterpreted as a change in identity, or vice versa. The cognitive distinction between these two components of face perception is reflected in the anatomical organisation of neural systems which underlie those two aspects of face processing (Haxby et al., 2000). Evidence for this division has come from neuropsychological studies of patients with face perception deficits (Young et al., 1995) and patients with prosopagnosia, from electrophysiological studies in non-human primates (Perrett et al., 1984) and from functional neuroimaging studies in humans (Haxby et al., 2000; Hoffman & Haxby, 2000).

Haxby et al. (2000) proposed a model of a distributed human neural system for face perception, which is based on the distinction between the system for identity perception and the system for perception of facial expressions (figure 1.4.1.2.). This model is divided into a core system, which deals with the visual analysis of the features, and an extended system, which is responsible for the further processing of faces. The core system consists of three neural areas in the extrastriate visual cortex: the lateral fusiform gyrus, the later inferior occipital gyrus, and the posterior superior temporal sulcus (STS). The region of the lateral fusiform gyrus seems to be involved more in the recognition of identity, whereas the STS seems to be more involved in the representation of the changeable aspects of face perception. The anatomical location of the inferior occipital gyrus suggests that it is involved in early visual processing of faces and provides input to both the lateral fusiform gyrus and the STS (Haxby et al., 2000). Evidence for a specialised face perception system

in the human brain has been obtained from neuropathological studies, electrophysiological studies, and from neuroimaging studies.

Patients with prosopagnosia have a selective impairment in the ability to recognise once familiar faces, whereas their object recognition remains relatively intact. Prosopagnosia is caused by lesions, usually bilateral, in the ventral occipitotemporal cortex (Haxby et al., 2002). Conversely, there have been reports of patients with a selective impairment of reading and object recognition, who perform normally in face recognition tasks (Kanwisher, 2000). This suggests a double dissociation between brain areas involved in object recognition and brain areas involved in face recognition.

Single cell recordings in non-human primates have identified neurons in the STS and the inferior temporal cortex in the macaque brain that respond selectively to faces (Perrett et al., 1982, 1984). Cells in those two areas have been shown to respond to different stimuli: cells in the STS respond primarily to different facial expressions, whereas neurons in the inferior temporal cortex respond primarily to different identities (Hasselmo et al., 1989). The corresponding human brain areas for these two regions are likely to be the STS and the lateral fusiform area.

Support for the functional dissociation between the STS and its role in the processing of changeable aspects of face perception and the lateral fusiform gyrus and its role in the processing of identity comes from an fMRI study (Hoffman & Haxby, 2000). This study showed that selective attention to eye gaze caused a stronger response in the superior temporal sulcus than did selective attention to identity, whereas selective attention to identity evoked a greater response in the lateral fusiform gyrus then did selective attention to eye gaze.

Functional neuroimaging studies of face perception consistently report activation in the lateral fusiform gyrus; this activation is frequently bilateral but has been found more consistently in the right hemisphere (Halgren et al., 2000; Haxby et al., 2000, 2002; Hoffman & Haxby, 2000; Kanwisher et al., 1997, 1999; Sergent et al., 1992). The activation in the lateral fusiform gyrus is greater in response to faces than in response to

other objects or non-sensical items, and has been termed the 'fusiform face area' (FFA) (Kanwisher et al., 1997, 1999). There is some debate whether the FFA is specialised for face perception (Kanwisher, 2000) or whether this area is involved in the processing of other visual stimuli as well (Gauthier et al., 2000). Kanwisher (2000) argues for a specialised face area, mainly based on neuropsychological evidence of the uniqueness of face processing, for example disproportionate disruption of face recognition caused by inversion of faces as compared to recognition of other inverted objects such as houses. Gauthier et al. (2000) on the other hand suggest that the face-responsive regions are specialised for visual expertise, based on an fMRI study demonstrating activation of the lateral fusiform gyrus in response to different birds and cars by subjects who were experts at bird and car recognition, respectively, although this activation was not as strong as the activation in response to faces. Other functional neuroimaging studies support the view that the FFA does not respond exclusively to faces, but rather responds maximally to faces, but also shows significant activation in response to other object categories (Haxby et al., 2000). On a neuronal level in non-human primates, neurons responding exclusively to human faces are intermixed in these regions with neurons that respond to other objects (Perrett et al., 1982).

Functional neuroimaging studies have also reported activation in the other two areas of the core face perception system, the STS and the inferior occipital gyrus (Halgren et al., 2000; Haxby et al., 2000; Hoffman & Haxby, 2000; Kanwisher et al., 1997; Puce et al., 1998). When the task consisted of passive viewing of faces or involved identity recognition the main focus of activation was in the FFA. However, if the task focussed attention on changeable aspects of the face, such as eye gaze or lip movement, the magnitude of activation in the FFA was reduced and instead activation was observed in the STS (Calder et al., 2002; Hoffman & Haxby, 2000; Puce et al., 1998).

Support for the core part of the face perception system has also come from evoked potential studies (Haxby et al., 2000; Kanwisher, 2000; Streit et al., 2000) and magnetoencephalography (MEG) (Streit et al., 1999).

Face perception does not stop at merely identifying the physical features of a face, but higher level processing of faces is important to facilitate verbal communication by lip reading, to determine someone's identity and therefore be able to access knowledge about them, to judge somebody's mood and infer their intentions, or to direct attention in the direction somebody else is looking. This more cognitive aspect of face perception is described in Haxby's model (Haxby et al., 2000) as the extended system. It includes brain areas, which are primarily involved in tasks other than face perception, but can be recruited to work with the extrastriatal face perception regions to assist with different aspects of face perception described above, such as lip reading to aid communication.

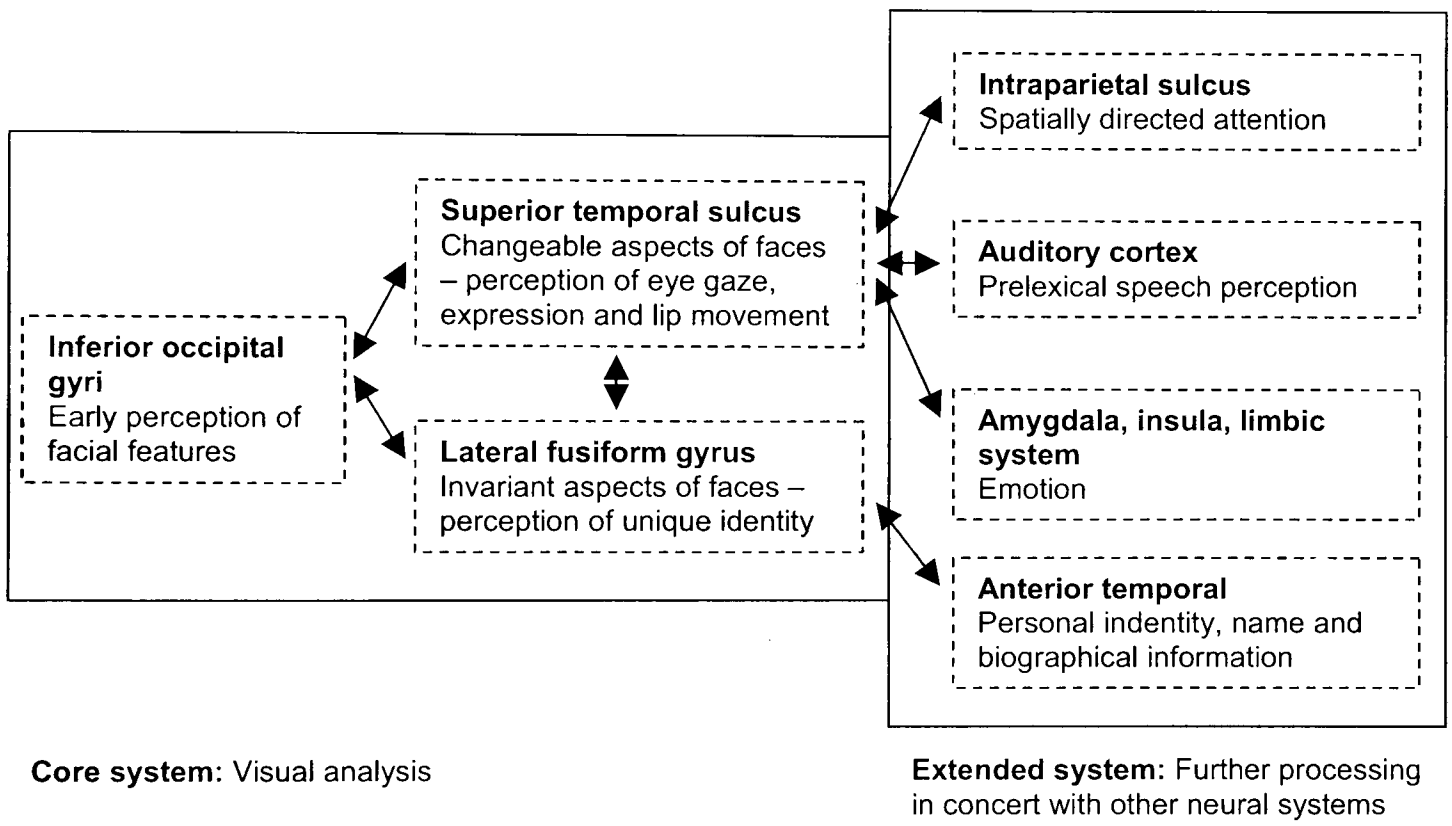


Figure 1.4.1.2: Model of the distributed human neural system for face perception. The model is divided into a core system consisting of extrastriate visual areas and an extended system, which acts in concert with the core system to facilitate higher level processing of face perception. Adapted from Haxby et al. (Haxby et al., 2000).

The extended system for face perception (see figure 1.4.1.2) includes the intraparietal sulcus, which is important for spatially directed attention, the auditory cortex, which is involved in lip reading, the anterior temporal cortex, which is concerned with accessing biographical information, and the limbic system, which is crucial for the perception of facial expressions of emotion (Haxby et al., 2000). As the focus of this thesis is on the

perception of emotions rather than on perception of other aspects of face perception I will not describe the extended system for face perception in detail. The neural correlates of facial expressions of emotion are described in detail in section 3.1.

1.4.2 The Neurobiology of Audition

1.4.2.1 Auditory Pathways

The structures of the inner ear provide the mechanisms for transforming sounds into neural signals. Sound waves cause the eardrum to vibrate, and those vibrations produce tiny waves within the inner ear's fluid that stimulate hair cells located on the basilar membrane within the cochlea. These hair cells are the primary auditory receptors, and they have receptive fields, which code for sound frequency. The output from the cochlea is projected to the cochlear nuclei in the brainstem and from there to the inferior colliculus. Efferents from the inferior colliculus project to the medial geniculate nucleus of the thalamus and from there to the auditory cortex. Both primary and secondary auditory cortex are located on the dorsal surface of the temporal lobes of the human brain. The most prominent projection, from the principal nucleus of the medial geniculate nucleus, extends to the primary auditory cortex (BA 41 and 42) on the transverse gyrus of Heschl. The auditory association cortex receives input from the primary auditory cortex and also from the auditory nuclei of the thalamus.

1.4.2.2 Neuroimaging of Human Affective Auditory Processing

In recent years there has been a wealth of neuroimaging studies of human auditory processes, from studies investigating the neural basis underlying basic auditory processes, such as pitch and intensity perception, to language perception and semantic memory (Engelien et al., 2001). In this chapter the focus will be on the neural correlates of the perception of affective auditory stimuli. There are many different affective auditory stimuli, ranging from music to words to non-verbal vocal stimuli such as laughter or crying. To ensure efficient communication it is important to be able to differentiate between socially relevant and socially irrelevant information. The ability to detect an emotion underlying either verbal or non-verbal communication is therefore essential to facilitate an appropriate behavioural response.

Some recent studies (Blood & Zatorre, 2001; Blood et al., 1999) have used pleasant and unpleasant music or environmental sounds to investigate the neural basis underlying affective auditory processing. Music can be a powerful elicitor of emotions even in the absence of the ability to identify or recognise a melody. This is surprising as music does not have an intrinsic biological or survival value unlike other stimuli that evoke emotions, such as smells, tastes, or visual stimuli such as faces. One study demonstrated a correlation of activity in paralimbic brain regions with unpleasant or mildly pleasant emotions elicited by varying degrees of dissonace. These regions included the parahippocampal gyrus, the orbitofrontal cortex, and the subcallosal and posterior cingulate gyri (Blood et al., 1999). Another study examining brain responses to intensely pleasurable music reported a correlation between intensity of pleasantness and changes in brain activity in the ventral striatum, insula, orbitofrontal cortex and amygdala. Interestingly, activation in the amygdala correlated negatively with the pleasantness of the music. It is suggested that intensely pleasurable music not only increases activation in brain regions associated with reward but simultaneously decreases activation in brain regions associated with negative emotions, such as the amygdala.

The neural responses to aversive auditory stimuli originating from the environment have also been investigated. Tinnitus-like auditory stimuli that were rated as unpleasant produced an increase in brain activation in bilateral primary and secondary auditory cortex, dorsolateral prefrontal cortex, inferior parietal cortex and limbic structures including the parahippocampal gyrus and the insula (Mirz et al., 2000). As the control condition was a silent baseline scan it is not surprising that activation in the auditory cortices was observed. In fact, it is difficult to conclude from this study whether any of the activated areas are involved in the processing of the emotional component of the stimuli or involved in auditory processing per se. Another study employed unpleasant environmental sounds like scraping metal, scratching nails on a blackboard and a dentist drill (Zald & Pardo, 2002). White noise was used as a control condition at a lower volume than the aversive sounds to avoid its being perceived as aversive. Activation was found in limbic and paralimbic structures including the amygdala, the putamen, nucleus accumbens, posterior insula, thalamus and the temporal pole, and also in secondary auditory cortex. The authors concluded that aversive sounds engage a distributed network of cortical and subcortical

brain regions, auditory cortex for processing the stimuli, limbic and paralimbic structures which are involved in the processing of the emotional component of the stimuli and in controlling appropriate autonomic and behavioural responses, such as the temporal pole, amygdala and insula, and brain structures which are involved in motor responses like muscle tension, such as the putamen and the cerebellum (Zald & Pardo, 2002). Noises of car crashes were contrasted with pleasant abstract sounds in a study by Frey et al. (2000). The only significant activation observed in response to the unpleasant sounds of the car crashes was in the bilateral caudal orbitofrontal cortex. This region was also activated in other studies (Blood et al., 1999; Zald & Pardo, 2002). It is likely that this orbitofrontal area works in conjunction with the temporal pole and the insula (Flynn, 1999) and is involved in extracting emotional significance from stimuli and initiating appropriate responses to them.

In addition to music and environmental sounds emotion can also be conveyed by the human voice, either by using an emotional tone of voice or by using emotional words. A voice-selective area in the human brain has been identified in the superior temporal sulcus (Belin et al., 2000). This region showed increased activation in response to both verbal and non-verbal vocal sounds as compared to environmental sounds. The voice does not just carry emotional information but also information about speaker identity. One study investigated brain regions involved in identification of the speaker and emotional tone of the voice (Imaizumi et al., 1997). It was found that different brain regions are involved in those two tasks. During the speaker identification task brain areas associated with face and object recognition including the temporal poles and parietal areas were activated. Emotion identification on the other hand recruited brain structures including the cerebellum and the inferior and middle frontal gyrus. Another study which utilized non-affective words spoken in different emotional tones investigated working memory associated with emotional vocal expressions (Raemae et al., 2001). Activation was found in inferior and middle frontal gyrus and the inferior parietal lobe. As in the previous study, no significant activation was found in structures of the limbic systems usually associated with emotional processes. It is possible that this is due to the explicit processing of the emotional stimuli. In both studies (Imaizumi et al., 1997; Raemae et al., 2001) subjects were required to identify the emotion conveyed and it has been shown that the activation of neural structures is dependent on

whether the task requires explicit or implicit processing of emotional stimuli (Critchley et al., 2000; Lange et al., 2003).

Neural activity in response to threat-related words has also been investigated. Compared to music, environmental sounds or the tone of voice conveying an emotion, this type of experiment relies on language processing. One study (Maddock & Buonocore, 1997) compared activation in response to neutral words and threat-related words in an fMRI block design study and reported posterior cingulate gyrus activation in response to threat-related words. Another study (Isenberg et al., 1999) used a modified Stroop task during a PET experiment. Subjects had to name the colour of either neutral or threat-related words. This study did not find posterior cingulate gyrus activation but reported amygdala activation instead. Although both studies examined brain activation in response to threat-related words they reported remarkably different results, which could be due to the different methodologies. The study using explicit emotion processing (subjects were asked to silently decide whether a word was neutral, pleasant or unpleasant) (Maddock & Buonocore, 1997) did not report amygdala activation whereas the study using implicit emotion processing (modified Stroop test) (Isenberg et al., 1999) did report amygdala activation. This again supports the hypothesis that activation in limbic structures which are involved in emotion perception such as the amygdala or the anterior insula are seen in response to implicit emotion processing but not in response to explicit emotion processing when the activation shifts to more neocortical areas such as frontal cortical areas which are associated with more cognitive functions, or in this case to posterior cingulate gyrus which has also been associated with cognitive functions such as memory (Maddock & Buonocore, 1997).

In order to avoid semantic processing as a confound while still investigating emotional communication some studies used non-verbal vocal stimuli, such as retching for disgust, laughter for happiness or crying for sadness. The first neuroimaging study using affective non-verbal vocal stimuli was by Phillips et al. (1998b). They investigated neural responses to both fearful and disgusting facial expressions and vocalisations. As predicted, amygdala activation was observed in response to fearful vocalisations, but no anterior insula activation was found in response to disgusting non-verbal vocal stimuli. A similar study

(Morris et al., 1999) using the same stimulus set (Scott et al., 1997) as Phillips et al. investigated brain areas involved in processing fearful, sad and happy vocalisations. When comparing emotional vocalisation (fearful, happy and sad taken together) to neutral they found activation in left middle temporal gyrus and superior frontal gyrus, and bilateral activation of the ventral prefrontal cortex and the anterior insula. In their analysis they focused on activation in response to fearful vocalisations and reported increased activation in the left anterior insula and middle temporal gyrus, and right superior frontal gyrus and ventral prefrontal gyrus, and a decrease of activation in right anterior insula and right amygdala. Using a regression analysis they found a significant fear-specific interaction between the left anterior insula and the right amygdala.

The most recent study to explore auditory perception of non-verbal vocal stimuli concentrated on laughter and crying (Sander & Scheich, 2001). Both conditions activated bilateral amygdala, with a more significant increase in activation in the right amygdala. Different control tasks (silence, subtraction of acoustically presented numbers, emotion induction or pitch-shift detection) did not affect the level of amygdala activation. This contrasts with the results of a study investigating the effect of task on brain activation in response to facial expressions of fear, which found amygdala activation only in response to a sex discrimination task but not in response to an explicit emotion processing task (Critchley et al., 2000; Lange et al., 2003). The bilateral insula was also found to be activated in response to both laughter and crying (Sander & Scheich, 2001). As this was a region of interest (ROI) analysis no other brain regions were investigated.

1.4.3 The Neurobiology of Olfaction

1.4.3.1 Peripheral Pathways

The olfactory sensory neurons are embedded in the olfactory epithelium, which is located in the back of the nasal cavity. Olfactory neurons are unusual as they are short-lived, with an average life span of one to two months, and are continuously replaced from basal stem cells underlying olfactory epithelium. Each olfactory neuron projects several cilia into the mucus coating the olfactory epithelium, and olfactory receptors are found on these cilia. Each odour receptor is thought to have its own unique binding properties to olfactory

molecules. It is suggested that each olfactory sensory neuron expresses only one receptor type and thus each neuron is very selective in the odorant molecules it can bind and in the subsequent transmission of information to the brain. The number of neurons that respond to a certain odorant varies with the concentration of the odorant. This might explain why a single odorant presented to human subjects at different concentrations can be perceived as being different odorants.

Receptor cells from the olfactory epithelium project across the cribriform plate and innervate the olfactory bulb, where they terminate in a single glomerulus. In the glomerulus the olfactory sensory neuron makes synaptic connections with three classes of neurons: mitral and tufted cells, and periglomerular interneurons. In the glomeruli a 100-fold decrease in the number of neurons transmitting olfactory information is achieved by thousands of receptor neurons converging onto only 20-50 mitral and tuftal cells, which project axons to the olfactory cortex (Buck, 2000). It is also suggested that neurons which express the same olfactory receptor terminate in the same few glomeruli. Some glomeruli seem to receive input from only a single olfactory receptor type.

1.4.3.2 Central Pathways

The axons of the mitral and tuftal relay neurons of the olfactory bulb project to the piriform cortex, olfactory tubercle, anterior olfactory nucleus, amygdala and entorhinal cortex (Greer, 1991), all of which constitute the primary olfactory cortex. Most parts of the primary olfactory cortex are located at the border of the anterior temporal lobes and the ventral frontal lobes. The olfactory system is, in fact, the only sensory system, which bypasses the thalamus and has direct projections to cortical areas. From the primary olfactory cortex both direct and indirect connections via the thalamus project to the orbitofrontal cortex. In addition, there are connections from the amygdala to the hypothalamus and from the entorhinal cortex to the hippocampus, which transmit olfactory information.

It has been suggested that the orbitofrontal cortex is the secondary olfactory cortex (Rolls et al., 1996), as the agranular insular transition cortex and a caudomedial part of area 13

receive direct input from the primary olfactory cortex (Carmichael et al., 1994) in the macaque monkey. Areas 14 and 25 have also been reported to receive olfactory input (Carmichael et al., 1994). Olfactory neurons in the orbitofrontal cortex have been shown to be able to learn associations between odours and rewards or punishments, and odour to taste associations (Rolls & Baylis, 1994).

Lesion studies in humans have highlighted the importance of the orbitofrontal cortex and temporal lobes in olfactory identification (Jones-Gotman & Zatorre, 1988) and discrimination tasks (Zatorre & Jones-Gotman, 1991), and the greater importance of the right than left anterior temporal cortex in olfactory memory (Rausch et al., 1977). Furthermore, the role of the amygdala in olfaction is indicated by the demonstration that amygdalotomy is a successful treatment for olfactory hallucinations in patients with seizure disorders (Chitanondh, 1966).

1.4.3.3 Neuroimaging of Human Olfaction

There have been relatively few neuroimaging studies of human olfaction. The first such study was performed in 1992 using PET (Zatorre et al., 1992) and reported bilateral activation of the piriform cortex and activation of the right mediolateral orbitofrontal cortex, corresponding to primary and secondary olfactory cortex respectively. Bilateral piriform and orbitofrontal cortex activations were also reported by Koizuka et al. (1994), who were the first to employ fMRI to investigate the neural correlates of human olfaction. Since then there has been some inconsistency with regard to piriform cortex activation across studies, with some studies reporting activation in piriform cortex (Bengtsson et al., 2001; Dade et al., 1998; Francis et al., 1999; Savic et al., 2000; Small et al., 1997a), but others finding activation in this area to be elusive (O'Doherty et al., 2000; Royet et al., 1999, 2001; Sobel et al., 1997; Yousem et al., 1997; Zald et al., 1998). An explanation for this inconsistency was proposed by Sobel et al. (2000). Using a block design during fMRI the time course of the response in the piriform was investigated and it was found that responses to olfactory stimuli habituate rapidly. This finding has been confirmed by Poellinger et al. (2001). They reported a 10s to 15s increase in activation after stimulus onset, which then rapidly diminished to sub-baseline levels. It was suggested that

hippocampus and anterior insula follow a similar time course during olfactory activation. The piriform cortex has also been associated with olfactory memory (Dade et al., 2002).

Activation in the orbitofrontal cortex in response to odours has been far more consistent (Zald & Pardo, 2000b). However, it is unclear what role lateralization of the activation plays, as some studies report right orbitofrontal cortex activation, some left, and some, bilateral activation (Brand, 2001). Activation in the right frontal lobe has been reported to be greater in females compared with males (Yousem et al., 1999), whereas another study investigating sex differences in response to odours does not report any orbitofrontal cortex activation (Bengtsson et al., 2001).

The roles of the piriform cortex, orbitofrontal cortex, amygdala and the entorhinal/hippocampal region in olfaction have been emphasised in a recent review of PET and fMRI studies of the human olfactory system (Zald & Pardo, 2000a). Although several neuroimaging studies have examined the neural correlates of olfaction per se (Koizuka et al., 1994; Royet et al., 1999; Savic et al., 2000; Sobel et al., 1998a, 1998b, 2000; Zatorre et al., 1992), only few studies have investigated the neural correlates of the perception of pleasant and unpleasant odours, with results that are as yet inconclusive.

Another region that has been consistently activated in most functional neuroimaging studies of olfaction is the insula (Francis et al., 1999; Fulbright et al., 1998; Poellinger et al., 2001; Savic et al., 2000; Small et al., 1997a; Zatorre et al., 1992). The majority of studies employed pleasant olfactory stimuli and reported either bilateral (Francis et al., 1999; Fulbright et al., 1998; Savic et al., 2000; Zatorre et al., 1992) or left anterior insula activation (Small et al., 1997a) in response to pleasant odours. The study by Fulbright et al. (1998) was the only one specifically examining the neural correlates of pleasant and unpleasant odours and they reported right insula activation in response to unpleasant odours.

Royet et al. (2000, 2001) reported activation of the left orbitofrontal cortex in response to the hedonic judgment of odours. Unlike most other studies, subjects had to make a conscious decision whether an odour was pleasant or unpleasant, and they were exposed to

both pleasant and unpleasant odours during the PET scan. A similar study reported right orbitofrontal cortical activation in response to both pleasantness and intensity judgments of odours (Zatorre et al., 2000). In the visual modality it has been shown that the type of task can influence the neural networks involved in emotion processing (Critchley et al., 2000), so it is important to take into account whether odours are presented in a passive perception paradigm or whether a cognitive task is required. It has also been suggested that the amygdala participates in the hedonic processing of olfactory stimuli, especially aversive olfactory stimuli (Birbaumer et al., 1998; Zald & Pardo, 1997). Upon re-analysis of their data, Zald & Pardo (2000b) also found left inferior insula activation in response to aversive odours, and no amygdala activation in response to pleasant odours. Contrary to this, a more recent fMRI study reported amygdala activation in response to intensity judgements of odours but not valence judgements (Anderson et al., 2003b). However, no amygdala activation was found in response to either pleasantness or intensity judgments of odours in another study (Zatorre et al., 2000). It is unclear from the studies to date which neural circuits are involved in the cognitive processing of olfactory stimuli, as there is some discrepancy between the results.

Regarding the neural correlates of perception of emotionally-salient odours, activation of the left medial frontal lobe, inferior frontal cortex, and bilateral insulae have been demonstrated during olfaction per se, with pleasant odours producing increased left insula activation compared with unpleasant odours (Fulbright et al., 1998). Another study has reported increased blood flow in left orbitofrontal cortex and bilateral amygdalae in response to perception of unpleasant (although not specifically disgusting) odorants (Zald & Pardo, 1997). Left lateral orbitofrontal cortex has also been shown to have increased activation in response to unpleasant odours regardless of intensity in another study (Anderson et al., 2003b), whereas right medial orbitofrontal cortex showed greater response to pleasant than unpleasant odours regardless of intensity (Anderson et al., 2003b). Perception of familiar odorants has been associated with right orbitofrontal cortical activation (Royet et al., 1999).

Although there are few studies investigating the affective component of olfaction, findings to date indicate that many of the brain regions associated with emotion perception are also

involved in the perception of olfactory stimuli: the orbitofrontal cortex, amygdala, and the insula. Since odours are rarely devoid of emotional salience, it is probable that the involvement of the orbitofrontal cortex, amygdala and insula demonstrated in the neural response to olfactory stimulation reflects, at least in part, processing of the emotional component of these stimuli.

Although previous studies have investigated neural responses to unpleasant or aversive olfactory stimuli no study has investigated disgusting stimuli. With the exception of pheromones, some of which are thought to convey fear, anger and sexual attraction in animals but possibly also in humans (Savic et al., 2001), disgust is the only emotion that can be directly translated from visual and auditory stimuli to olfaction. Some stimuli used in previous studies might have been perceived as disgusting by subjects, but this was not looked at directly. Early studies relied on very primitive stimulus delivery which could not control the amount of odour delivered (Koizuka et al., 1994), but even studies employing a very sophisticated olfactometer had limitations, such as only two available channels in the olfactometer (Francis et al., 1999; O'Doherty et al., 2000). In this thesis, neural responses to disgusting olfactory stimuli will be investigated and compared to neural responses to pleasant and unpleasant but not disgusting olfactory stimuli.

1.4.4 The Neurobiology of Gustation

1.4.4.1 Peripheral Pathways

The receptors responsible for the peripheral encoding of taste stimuli are taste cells. These are located on the surface of the tongue (some are also located on the palate, the pharynx, the epiglottis and the upper third of the oesophagus) and are clustered into taste buds. Taste cells are known to be responsive to five prototypical tastes: sweet, sour, salty, bitter, and the recently discovered umami, which corresponds to the taste of protein. The receptor cells transduce soluble chemical stimuli into electrical signals, some involving specific receptors for specific tastes. Taste buds of the anterior two thirds of the tongue are innervated by gustatory fibres from the chorda tympani, whereas the posterior third of the tongue is innervated by the lingual branch of the glossopharyngeal nerve (Buck, 2000). These nerves terminate in the rostral nucleus of the solitary tract (NTS), and then project on to the

parvicellular division of the posteromedial thalamic nucleus (VPMpc) where taste responsive neurons have been found (Pritchard et al., 1989).

1.4.4.2 Central Pathways

The taste thalamus (VPMpc) projects directly to a region in the anterior insula/frontal operculum (Pritchard et al., 1986). This region is the first area to receive monosynaptic efferents directly from the thalamus and has therefore been described as the primary taste cortex. There are few lesion studies examining taste perception in subjects with lesions in higher cortical areas rather than the brain stem or midbrain. The anterior insula/frontal operculum was first implicated in gustatory function when Bornstein observed in 1940 that patients with bullet wounds in that area experienced ageusia. Some epilepsy patients experience gustatory hallucinations, which can be induced by stimulation of the parietal or rolandic operculua (Hausser-Hauw & Bancaud, 1987).

Neurophysiological investigations of responses of single neurons to taste stimuli have helped describe primary and secondary gustatory cortex in the non-human primate brain.

1.4.4.3 Neuroimaging of Human Gustation

Much less is known about taste processing in the human brain. Prior to functional neuroimaging studies, no precise knowledge existed of the extent and exact location of the primary and secondary taste cortices or of the degree to which the findings from non-human primates were generalisable to humans. Within the past decade a relatively small number of human neuroimaging studies investigating neural correlates of taste perception have been carried out. Most of these studies have used positron emission tomography (PET) and have employed simple experimental protocols to produce taste stimulation.

In a comprehensive review of neuroimaging studies investigating neural substrates for taste perception (Small et al., 1999), the insula, parietal and frontal opercula, and the orbitofrontal cortex were shown to play important roles, predominantly within the right hemisphere. The authors have suggested that orbitofrontal activity may be dependent upon

motivational features of specific taste tasks, whilst the right hemisphere predominance for taste perception may be a result of the specialisation of the left hemisphere for language. One study has also reported activation of the anterior cingulate gyrus and thalamus in addition to the areas mentioned above (Faurion et al., 1999). Others have emphasised the importance of activation in the insula and perisylvian region, and reported functional lateralization of taste perception related to handedness in the inferior part of the insula (Faurion et al., 1999).

With the exception of Zald et al. (1998, 2002) and O'Doherty et al. (2001b), the representations of pleasant and aversive taste have not been compared within the same subject group in a neuroimaging study. Zald (1998), using PET, found that a region of the left orbitofrontal cortex and the amygdala were activated by aversive taste, but not in response to pleasant taste. In a later study (Zald et al., 2002) Zald used sucrose solution instead of chocolate and reported amygdala activation in response to sucrose solution but only when compared to rest and not when compared to tasting water. O'Doherty on the other hand found bilateral activation of orbitofrontal cortex, amygdala, and insular cortex/frontal operculum in response to both pleasant and unpleasant tastes. There is some evidence that orbitofrontal cortical areas activated by pleasant and unpleasant tastes are topographically separate (O'Doherty et al., 2001b). One difference between the studies is that O'Doherty used artificial saliva as the control condition whereas Zald used water. Water has been shown to weakly activate the amygdala (Zald & Pardo, 2000a) and can be perceived as pleasant rather than neutral (Brunstrom et al., 1997) and is therefore not a good control stimulus.

So far no effective way of delivering gustatory stimuli in a neuroimaging, especially fMRI, environment has been developed. Some previous studies employed rather crude delivery of stimuli, for example feeding subjects pieces of chocolate (Zald et al., 1998), which causes head movement problems with chewing and swallowing. Many studies exploring the neural correlates of taste have been performed using PET, which limits the spatial resolution of the results (Small et al., 1999).

The findings of these studies indicate that there is a significant overlap between regions important for perception of distinct flavours and those involved in emotion perception. Flavours, like odours, frequently have an emotional significance, and it is probable that they, too, are processed to a large extent in terms of their emotional component.

As for olfaction, disgust is the only emotion that can easily be directly translated from vision and audition into the gustatory modality. Previous studies have investigated the neural correlates of unpleasant or aversive gustatory stimuli but no study to date has directly investigated the neural response to disgusting tastes. In this thesis the neural correlates of disgusting tastes will be investigated and compared to the neural responses to pleasant and unpleasant tastes.

1.5 Summary

This introductory chapter has introduced the main theme of the thesis, which is the neural networks underlying the perception of emotions, especially disgust, in four sensory modalities: vision, audition, olfaction and gustation. The main theories of emotion have been reviewed in section 1.2, ranging from James 1884 "feeling" theory to Ekman's argument for basic, separate emotions. This theory will be followed in this thesis, in chapter 3 the neural correlates of the perception of anger, fear, disgust and sadness in the visual and auditory modality are investigated, with a focus on the perception of disgust in subsequent chapters.

The neural basis of emotion perception has been reviewed in section 1.3, closely following Phillips' division of emotion perception into three distinct processes: the identification of emotionally-salient stimuli, the production of an emotion in response to the stimuli, and the regulation of the affective state and emotional behaviour. The work in this thesis is mainly concerned with the identification of emotionally salient stimuli in the four sensory modalities under investigation. Entering into an affective state in response to those stimuli cannot be excluded. However, control or regulation of the affective state or any emotional behaviour was not investigated in this thesis.

In section 1.4 the neurobiology of vision, audition, olfaction and gustation has been reviewed. As these are the modalities under investigation, the pathways for stimulus perception have been described and functional neuroimaging studies of perception discussed.

The following chapter introduces the method used in this thesis: functional magnetic resonance imaging. Physiological issues concerning the BOLD (blood oxygenation level dependent) response are discussed. The way the data was acquired is described, as is the main analysis method common to all experimental chapters.

Chapters 3-6 describe the experiments performed. Chapter 3 deals with the perception of anger, fear, disgust and sadness in the visual and auditory modality. Chapter 4 further analyses the results from chapter 3 but with an emphasis on the visual modality, and methodological issues of experimental design and how this can influence results are explored, using the perception of facial expressions of disgust as the example. The perception of disgust in the olfactory modality is studied in chapter 5, and chapter 6 deals with the perception of disgust in the gustatory modality. In chapter 7 the results from the previous experimental chapters are compared with previous results by Phillips et al. (1997, 1998b, 2000). In the last chapter, chapter 8, all the findings are integrated into the theoretical framework, and issues affecting emotion perception and methods used to investigate this are discussed.

Chapter 2

Methods

This chapter will provide a brief overview of the principal technique that will be used in this thesis, functional magnetic resonance imaging (fMRI), together with a brief discussion of issues and problems encountered when applying neuroimaging techniques to multiple sensory systems.

2.1 Introduction to functional magnetic resonance imaging

Magnetic resonance imaging is based on the fact that some atomic nuclei, such as the hydrogen nucleus, possess a property called 'spin' or magnetic momentum, which means that they can be considered to be similar to tiny dipole magnets. Each nucleus has an orientation or axis around which it precesses or spins. Outside a magnetic field the orientation of the nuclei within a tissue are essentially random. However, when tissue is placed into a magnetic field such as the strong homogeneous magnetic field of a magnetic resonance (MR) scanner, the nuclei become oriented with respect to the prevailing magnetic field. In order to produce an image, other magnetic fields are applied in orthogonal directions to the sample. This is achieved by using a radio frequency (RF) pulse, which induces a precession in the protons, which is around the same direction as the applied field. Once the RF pulse is turned off, the phase coherence of the nuclei gradually decays and reverts to the previous equilibrium.

Two principle parameters are used to describe the magnetic resonance signal (Cohen & Bookheimer, 1994). The rate at which the MR signal approaches equilibrium, T_1, is one, the other parameter, T_2, is the rate at which the MR signal decays after an RF pulse, by each spin interacting with others nearby. In addition to this, local variations in magnetic field strength cause the protons to precess at slightly different frequencies and they become out of phase with one another. These variations in field strength are caused by imperfections in the main magnetic field or by tissues or particles with different magnetic

susceptibility, and they result in signal loss. This signal decay rate, T_2*, is the variable that is used to weight images in fMRI. The echo time is the time between the first RF pulse and the measurement - by waiting a further short period, differences in the decay rates become apparent as differences in the intensity of the MR signal, as the T_2* weighting increases.

This susceptibility to local magnetic field inhomogeneities is exploited in functional magnetic resonance imaging using one of a number of fast imaging pulse sequences, such as echo-planar imaging (EPI). By use of specialist gradient coils with very rapid switching frequencies, EPI enables an image to be sampled following the delivery of a single excitation pulse, and therefore allows a faster acquisition rate than conventional pulse sequences. Depending on the spatial resolution used, EPI can enable sampling of the whole brain in less than 3 seconds.

2.1.1 The BOLD response

Functional imaging uses the fact that oxyhaemoglobin and deoxyhaemoglobin differ slightly in their magnetic susceptibility (Ogawa et al., 1990). As the signal decay rate of protons in the vicinity of deoxyhaemoglobin is faster than that of oxyhaemoglobin because it is paramagnetic, the effects of blood oxygenation on T_2* can be exploited. Deoxyhaemoglobin is said to be an endogenous contrast agent. This is termed the BOLD (blood oxygenation-level dependent) method.

The physiological basis of the BOLD response is currently not fully understood. However, it is known that BOLD contrast depends on the level of oxygenation in the venous vessels (figure 2.1.1a), which varies because changes in brain activity are accompanied by changes in regional blood flow, causing a local increase in oxygen levels. There is an initial decrease in oxygenation when consumption exceeds the supply. This is followed by an increase in blood flow to the area, which is greater than the demand for oxygen, which causes the oxyhaemoglobin concentration to rise and therefore leads to an increase in signal intensity. Given that the positive BOLD signal is dependent on regional blood oxygenation increases and not immediately caused by neural metabolism induced changes in deoxyhaemoglobin

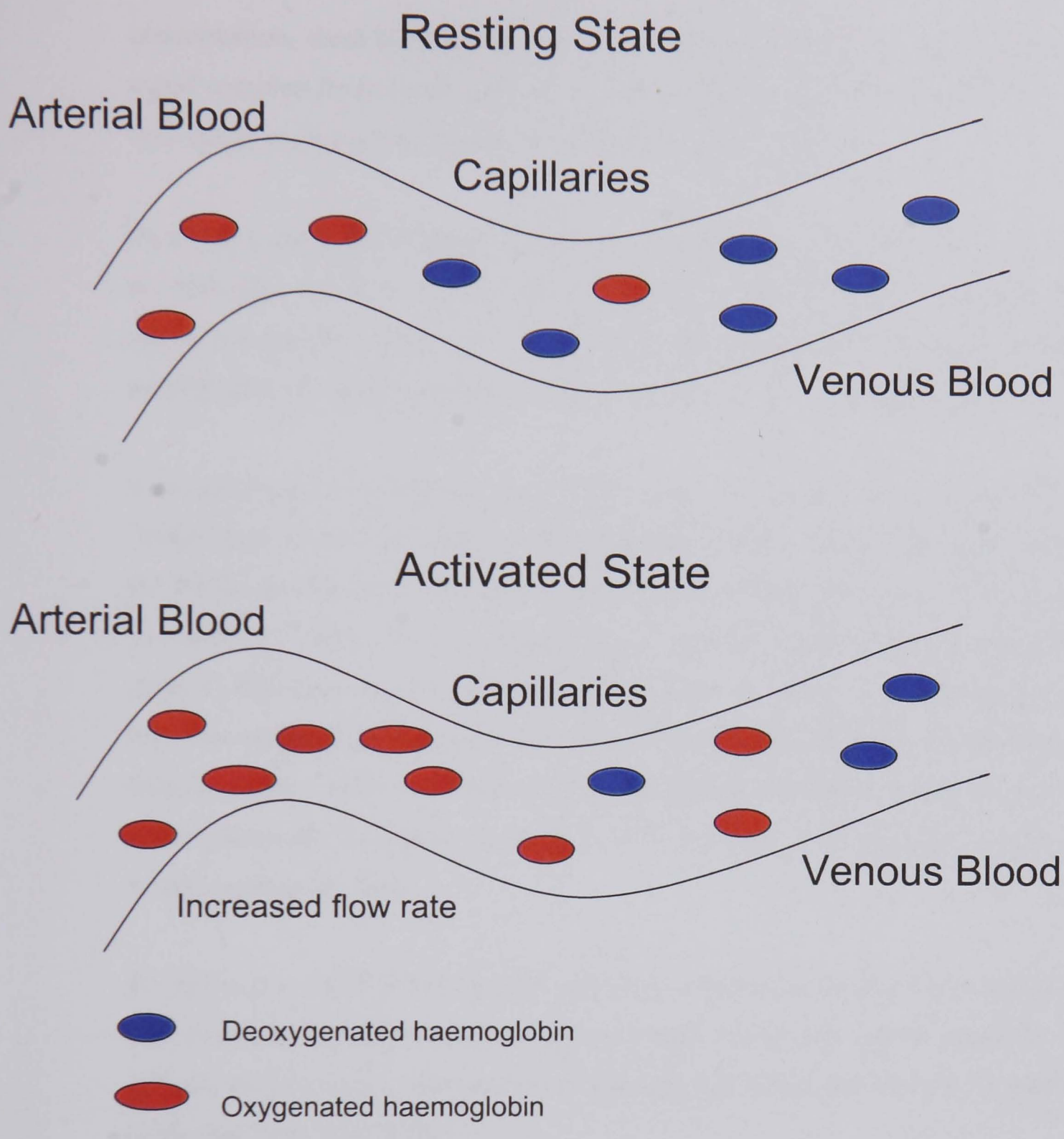


Figure 2.1.1.a: Physiological basis of BOLD signal
Illustration of the presumed physiological basis of the positive BOLD signal. In the activated state, the increased blood flow results in a greater quantity of oxygenated blood arriving in the blood vessels feeding the area of neural activity, and consequently the ratio of oxygenated to deoxygenated blood is higher than in the resting state. This effect is detected as a positive BOLD signal, as oxyhaemoglobin gives a greater T2* weighted MR signal than deoxyhaemoglobin. This diagram has been adapted from a lecture series given by Dr. P. Jezzard. (http://www.fMRIb.ox.ac.uk/~peterj/lectures/hbm_2/sld020.htm)

concentration, there is an appreciable delay between the neural activity and the observed signal resulting from the increase in blood oxygenation, as it takes about 4 to 6 seconds for the oxyhaemoglobin concentrations to build up to a maximum.

As a result, the positive BOLD signal corresponding to any neural activation only reaches its peak after this time. As temporal resolution and signal detection improve, it will be possible to use the 'initial dip' as a more accurate measure of neural activity. When neural activity ends, the signal returns to baseline after about 8 to 11 seconds (figure 2.1.1b).

A recent paper by Logothetis et al. (2001) shows that a spatially restricted increase in the fMRI signal is directly related to an increase in neural activity. The major determinant of the fMRI signal turned out to be the local field potentials (LFP) rather than single- or multi-unit spiking activity. This was interpreted as the fMRI signal predominantly reflecting the input to that area and the corresponding changes in information processing, rather than neuronal output from that area. However, although the LFP was a better predictor of the BOLD response than multi-unit activity, the difference between them was not large. As action potentials can also contribute to LFP, the local field potential reflects more than merely input to the area.

Therefore it is likely that both LFP and multi-unit activity contribute to the BOLD signal (Bandettini & Ungerleider, 2001). Some studies report that action potentials correspond with the BOLD signal, whereas others conclude that synaptic activity is the better predictor (Arthurs & Boniface, 2002).

It is important to note that the signal-to-noise ratio during direct recordings of neural signals is much greater than the corresponding fMRI signal-to-noise ratio (Raichle, 2001). Therefore the absence of a detected BOLD change in a particular area of the brain does not automatically mean that no information processing is taking place.

Another issue with the BOLD response concerns the extent to which the location of the observed activation corresponds to the spatial location of the underlying neural activity. The complicated coupling of the BOLD response with rCBF increases means that the

complicated coupling of the BOLD response with rCBF increases means that the BOLD response does not only occur at capillary level but also at large draining veins which can be downstream from the actual focus of the activation site (Arthurs & Boniface, 2002). There is reason for caution when interpreting the precise spatial location of an observed BOLD response in terms of the underlying neuronal responses.

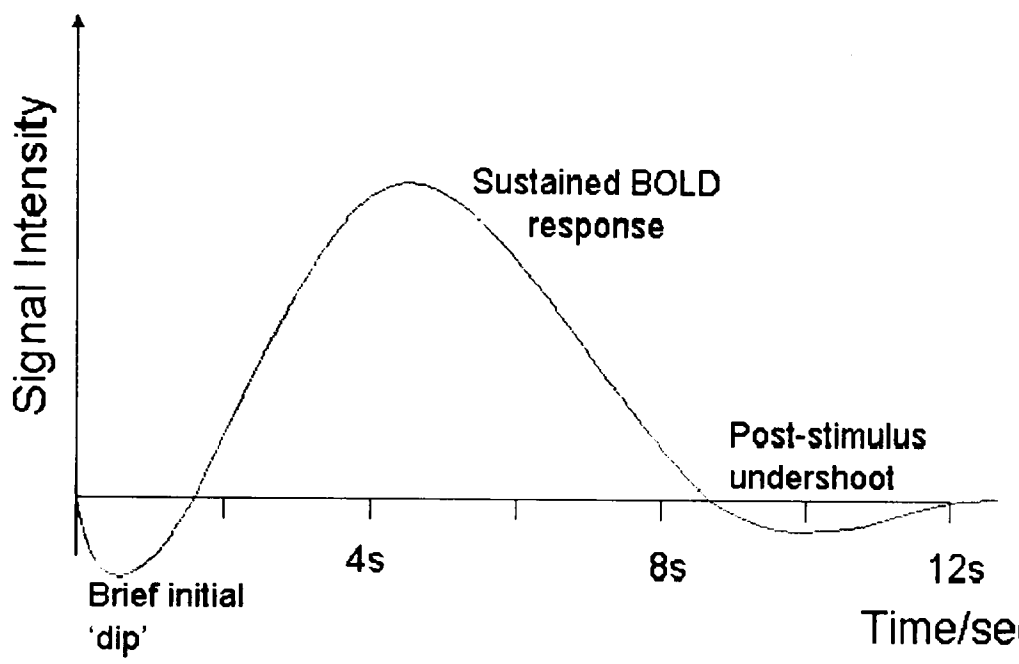


Figure 2.1.1b: Time course of BOLD signal
Illustration of the time course of the BOLD signal in response to a brief stimulus given at time 0s. During the initial transient 'dip' in signal intensity the oxygen consumption exceeds the supply. This is followed by an increase in blood flow to the area, which is greater than the demand for oxygen, hence an increase in oxyhaemoglobin concentration and therefore an increase in signal intensity. Before returning to baseline there is an undershoot in signal intensity. This is due to the flow having returned to normal but a slow recovery in cerebral blood volume means an effective increase in deoxyhaemoglobin.

A further question concerns whether the BOLD signal change only reflects excitatory processing or whether it can also reflect inhibitory processes. Inhibitory synaptic activity might modulate the BOLD response by altering metabolic demand or by influencing net spiking activity. As recycling of inhibitory neurotransmitters also requires energy, this might

processing does not produce a change in the BOLD signal, and others claiming that it does (Arthurs & Boniface, 2002).

2.2 Data acquisition

The fMRI technique used in this thesis measures signal changes in the whole brain, using echo-planar imaging at a field strength of 1.5 Tesla. There is an important technical issue, which needs to be mentioned as it has implications for the data analysis. This is signal dropout due to magnetic susceptibility in the orbitofrontal cortex and anterior temporal lobes.

As described above, BOLD imaging is based on detecting the small changes in magnetic susceptibility that are introduced by transient changes in blood oxygenation. However, this imaging technique is also sensitive to a less advantageous source of field inhomogeneities in the brain caused by differences in magnetic susceptibility between different adjacent tissues. The region with the most prominent magnetic field inhomogeneities is the orbitofrontal cortex, due to the susceptibility differences between this region and the adjoining sinuses. This can produce two main effects. The first effect is substantial signal loss from this area, which manifests itself as a hole in the image where the orbitofrontal cortex should be (figure 2.2). A second effect is geometric distortion, as unanticipated inhomogeneities in the magnetic field can interfere with the accuracy of the frequency encoding and result in the construction of a distorted image. This signal drop-out can be minimised by a number of measures, such as data acquisition in coronal slices, using a shortened echo time, or acquiring slices with reduced in-plane voxel size.

However, as the focus of this thesis was not on the orbitofrontal cortex but on the whole brain a conventional data acqusition sequence was chosen. Data acquisition remained the same for all experiments in order to allow comparison of areas of activation between the studies performed for this thesis and comparison with previous studies (Phillips et al., 1997, 1998b) that had been performed at the Institute of Psychiatry and on the results of which the idea for this thesis was based.

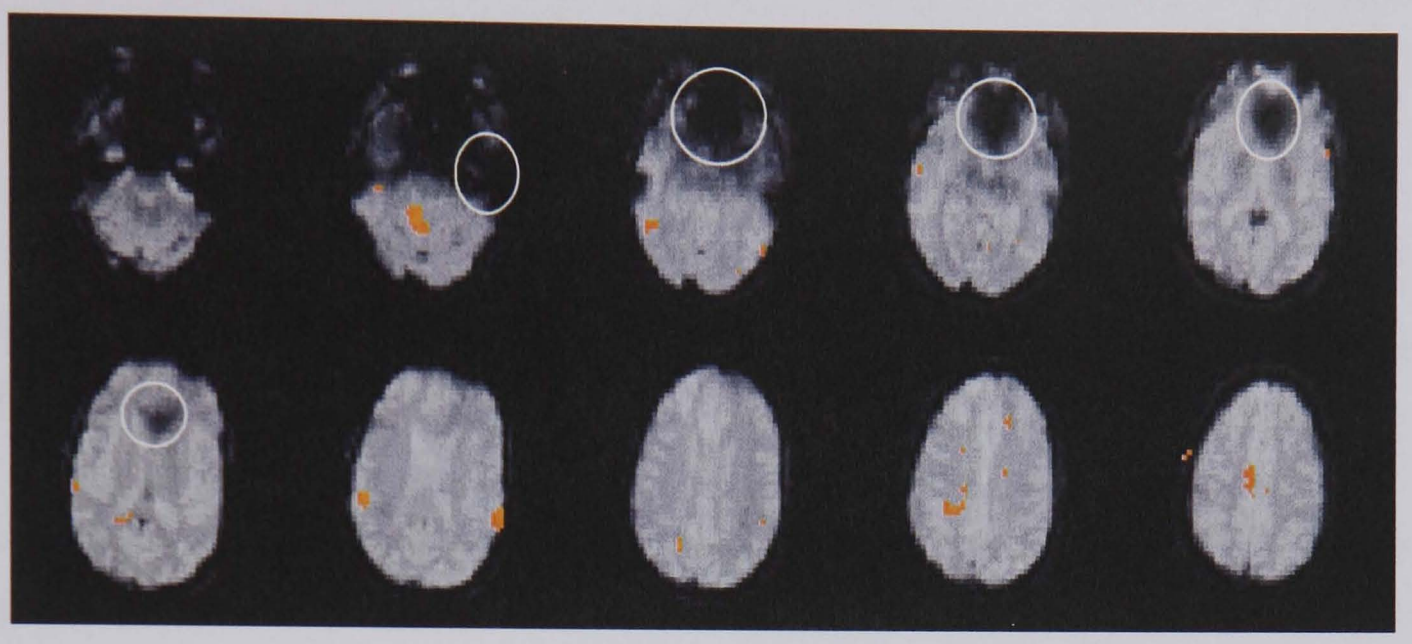


Figure 2.2: Example of signal dropout
This is an individual brain activation map of one subject prior to being mapped into Talairach space. The signal dropout in the orbitofrontal lobe and inferior temporal lobe is encircled in white.

Gradient-echo echoplanar MR images were acquired using a 1.5 Tesla GE Signa Neuro-optimised System (General Electric, Milwaukee, WI, USA) fitted with 40 mT/m high speed gradients at the Maudsley Hospital, London. Foam padding and a forehead strap were used to limit head motion. Daily quality assurance was carried out to ensure high signal to ghost ratio, high signal to noise ratio and excellent temporal stability using an automated quality control procedure (Simmons et al., 1999). A quadrature birdcage head coil was used for RF transmission and reception.

In each of 14 near-axial non-contiguous planes parallel to the inter-commissural (often referred to as AC-PC, anterior commisure-posterior commisure) plane, 100 T2*-weighted MR images depicting BOLD contrast (Ogawa et al., 1990) were acquired over 5 minutes (for each task): TE (echo time) = 40 ms, TR (repetition time) = 3000 ms, theta = 90 degrees, in-plane resolution = 3.75 mm, slice thickness = 7 mm, interslice gap = 0.7 mm. This EPI dataset provided complete coverage of the temporal lobes (including hippocampus and amygdala) and almost complete coverage of frontal, occipital and parietal lobes (Simmons et al., 1999). At the same session, a 43-slice, high-resolution inversion recovery echoplanar image of the whole brain was acquired in the AC-PC plane: TE = 73 ms, TI

(inversion time) = 180 ms, TR = 16,000 ms, in-plane resolution = 1.5 mm, slice thickness = 3 mm.

2.3 Experimental Design

Two different experimental protocols are common in current fMRI studies: block design fMRI and event-related fMRI.

The concept behind the block design is that the experimental stimulation or task is applied during a fixed number of scans which constitute the ON period, and in addition, another series of scans are collected during which the subjects rest or participate in a control condition, this constitutes the OFF period. A number of such ON/OFF blocks are then collected in order to provide enough statistical power to detect significant signal changes by comparing volumes collected during the ON period with volumes collected during the OFF period.

It is important to choose an appropriate control condition as the choice of this can greatly influence results (Newman et al., 2001). The most commonly used control conditions include rest, a passive task, or a task-related condition. During a rest condition, however, the brain is never literally 'resting', and it has been suggested that conceptual and linguistic thoughts arise (Newman et al., 2001). This makes it difficult to be certain what is actually happening during this control condition. A similar limitation occurs with a passive control task, although the volunteers are presented with stimuli it is difficult to determine what they are attending to. A task-related control condition is thought to separate specific mental operations and is based on the reasoning that it is possible to insert or omit certain processes that are interlinked or dependent on each other. This is also called cognitive subtraction. It has, however, been shown that it is almost impossible to alter processes or components of processes without affecting other processes (Newman et al., 2001). As can be seen there are disadvantages to all three types of control conditions. In this thesis both passive control conditions and task-related control conditions are used as the rest control condition was deemed too uncertain. Control conditions will be described in more detail in each experimental chapter.

In contrast to the block design, event-related designs are based on the principle that the fMRI response to a single event can be detected. The idea is that the temporal resolution of fMRI is sufficient to measure any signal change in response to an event such as a stimulus being presented. There is little time constraint when this stimulus event occurs as it does not have to be repeated at fixed intervals or be presented for a fixed amount of time, there only have to be sufficient number of repetitions of the stimulus event to enable enough statistical power to reliably detect significant changes in brain activity. In practical terms this often means experiments are longer than with the block design to ensure adequate statistical power. The main advantage of the block design compared to other designs such as an event-related design is its robustness.

Each subject performed four 5-minute experiments. Each experiment comprised five cycles of periodic alternation between 30s epochs of emotional stimuli (ON) and neutral stimuli (OFF). The experimental design for each study for this thesis is described in more detail in the experimental chapters.

2.4 Data Analysis

In order to produce activation maps from fMRI images, a number of processing steps must be carried out. The aims of these processing steps are essentially to eliminate or reduce noise which is present in the data and unrelated to the experimental manipulation, thus introducing confounds in the data which affect the statistical interpretation of the results. Slight subject movement during data acquisition can cause changes in T2*-weighted signal intensity unrelated to changes in cerebral blood flow. Any effects of subject motion during image acquisition were corrected prior to any further analysis by realignment by tricubic spline interpolation followed by regression of each realigned fMRI time series on a second order polynomial function of lagged and concomitant positional displacements of the subject's head (Bullmore et al., 1996; Brammer et al., 1997). Following movement correction, the data were analysed using generic brain activation mapping (GBAM) software (Brammer et al., 1997). This is a non-parametric analysis which avoids some of the main confounds of the more traditional parametric analysis techniques. As the number

of subjects tends to be small (typically less than 10) any outliers can disproportionately affect the mean of any individually estimated test statistic, GBAM therefore uses the median as a test statistic. Additionally, the distributional distortions caused by image processing may influence the validity of parametric hypothesis testing.

2.4.1 Generic brain activation mapping

Periodic change in T2*-weighted signal intensity at the (fundamental) experimentally determined frequency of alternation between A and B conditions (= 1/60 Hz in both tasks) was estimated by an iterated least squares fit of a sinusoidal regression model to the fMRI time series observed at each voxel (Bullmore et al., 1996). This model included sine and cosine waves at the fundamental AB frequency of the experimental input function, parameterised by coefficients {g, d}. The power of periodic response to the input function was estimated by (g^2+d^2); and this fundamental power divided by its standard error yielded a standardised test statistic, the fundamental power quotient (FPQ), at each voxel. Parametric maps representing FPQ observed at each intracerebral voxel were constructed. In order to sample the distribution of FPQ under the null hypothesis that observed values of FPQ were not determined by experimental design (with few assumptions), the 99 images observed in each anatomical plane were randomly permuted and FPQ was estimated exactly as above in each permuted time series. This process was repeated 10 times, resulting in 10 permuted parametric maps (for each subject at each plane) of FPQ estimated under the null hypothesis that FPQ is not determined by experimental design (Bullmore et al., 1996).

Observed and randomised maps of FPQ were then registered in the standard space of Talairach & Tournoux (1988). This was achieved in two stages, using realignment algorithms similar to those previously used for movement correction. First, the set of FPQ maps observed in each subject is registered with that subject's high resolution EPI dataset; then registered and rescaled relative to a Talairach template image. Identical transformations were applied to the randomised FPQ maps obtained for each subject. After spatial normalisation, the observed and randomised FPQ maps from each subject were identically smoothed with a Gaussian filter (full width half maximum = 11mm) to

accommodate variability in gyral anatomy and error of voxel displacement during normalisation. Generic activation was then robustly decided by computing the median value of FPQ at each intracerebral voxel of the observed parametric maps, and comparing it to a null distribution of median FPQ values computed from the randomised parametric maps, using a one-tailed t-test. If the observed median FPQ exceeded the critical value of randomised median FPQ, then that voxel was considered generically activated with voxel-wise probability of false positive activation $P \leq 0.004$. This is the equivalent of 50 false positive voxels per image, which consists of a search volume of approximately 12000 voxels. The results are therefore typically only reported for clusters containing 5 activated voxels or more, unless there was very little overall activation, or activation of less than 5 voxels in an area predicted by an a priori hypothesis. Generically activated voxels were coloured and superimposed on a gray scale Talairach template used for spatial normalisation, to create generic brain activation maps (GBAMs) (Brammer et al., 1997). 'Generic' is used to mean activation demonstrated over all subjects in a group.

Some data for which subject movement in the scanner caused problems during the analysis were analysed with a more recent version of GBAM, which has improved movement correction. The responses at each voxel were analysed by regressing the corrected time-series data on a linear model produced by convolving each contrast vector to be studied with two Poisson functions parameterising haemodynamic delays of 4 and 8 seconds (Bullmore et al., 2001). Following least squares fitting of this model, a goodness of fit statistic composed of the ratio of model to residual sum of squares was calculated (Edgington, 1995) for each contrast. The distribution of the same statistics under the null hypothesis of no experimental effect was then calculated by wavelet-based resampling of the time-series at each voxel and refitting the models to the resampled data (Bullmore et al., 2001). An experimentally derived null distribution of the goodness of fit statistic was then derived by following this procedure ten times at each intracerebral voxel and combining the resulting data. This method has been shown to give excellent control of nominal type I error rates in fMRI data from a variety of scanners. Activations for any contrast at any required p value can then be determined by obtaining the appropriate critical values from the null distribution (Bullmore et al., 1996). Generic group activation maps were constructed by mapping the observed and randomised test statistics for each individual into the standard

stereotactic space of Talairach and Tournoux (1988) and computing and testing median activation maps as previously described (Brammer et al., 1997).

Additional methods of data analysis were used for some of the experiments and are described in detail in the experimental chapters.

Chapter 3

Neural responses to auditory and visual presentations of anger, disgust, fear and sadness

3.1 Introduction

The recognition of facial expressions of emotions seems to be an important prerequisite for the social communication of humans, as facial expressions often indicate internal emotional states and intentions (Darwin, 1872/1998; Ekman, 1992). Observing emotional expressions in others can lead to facial mimicry and emotional contagion, the induction of observed emotion in the viewer. This is an automatic and largely unconscious process, thought to be important for communicating affective states and empathy. Facial expressions of happiness and sadness were found to be particularly effective in inducing the emotion in the viewer (Hess & Blairy, 2001; Wild et al., 2001). The neural structures underlying the perception of emotions have been investigated in lesion and neuroimaging studies.

Lesion studies have indicated the importance of limbic and paralimbic structures such as the orbitofrontal cortex, insula, amygdala, hippocampus and parahippocampal gyrus, temporal pole and retrosplenial cingulate gyrus, as well as transmodal cortical sites for the recognition and identification of emotions from facial expressions (Weniger & Irle, 2002). Lesion studies have also shown a double dissociation between (1) patients with amygdala lesions who are more impaired in recognising facial expressions of fear than of other emotions (Adolphs et al., 1994, 1996; 1999; Calder et al., 1996; Young et al., 1995, 1996), and (2) patients with or carriers of the gene for Huntington's disease and a patient with lesions of the insula and striatum who are more impaired in recognising facial expressions of disgust than facial expressions of fear (Calder et al., 2000; Gray et al., 1997; Sprengelmeyer et al., 1996). Patients suffering from obsessive-compulsive disorder also display severe deficits in recognizing facial expressions of disgust (Sprengelmeyer et al., 1997).

This double dissociation between different lesion sites and deficits in recognising specific emotions has been supported by several functional imaging studies of the perception of facial expressions of emotion. These studies have reported amygdala activation in response to facial expressions of fear (Breiter et al., 1996; Morris et al., 1996; Phillips et al., 1998b; Whalen, 1998) but anterior insula activation in response to facial expressions of disgust (Phillips et al., 1997, 1998b, 1999; Sprengelmeyer et al., 1998). These results suggest that the processing of fearful and disgusted facial expressions are mediated by at least partially dissociable neural systems, and it is therefore likely that distinct neural activation patterns underlie the processing of other basic emotions, such as happiness, anger and sadness.

Early functional imaging studies have concentrated on the perception of facial expressions per se rather than individual emotions. They have implicated the right hemisphere (Gur et al., 1994), bilateral cingulate cortex (Sergent et al., 1994), and right anterior cingulate and bilateral inferior frontal cortex (George et al., 1993) in recognition of positive and negative facial expressions. Emotion discrimination from facial expressions has been associated with amygdala and hippocampal activation (Gur et al., 2002). More recent neuroimaging studies have focussed on the neural substrates underlying different emotions. These have been investigated using facial expressions of emotions, emotional scenes, emotion induction and auditory stimuli. Two extensive recent reviews by Phan et al. (2002) and Wager et al. (2003) performed meta-analyses of emotion activation studies in functional neuroimaging.

There seems to be a network of neural regions involved in the perception of emotions per se, including limbic areas, cingulate cortex and inferior frontal cortex. It has been suggested that the output of specific pathways for individual emotions could converge on the inferior frontal gyrus for further information processing (Berthoz et al., 2002; Gorno-Tempini et al., 2001; Sprengelmeyer et al., 1998). However, the only neural region that was commonly activated across studies included in the meta-analysis (Phan et al., 2002) was the medial prefrontal cortex, which had previously been thought to be involved in emotional decision making (Damasio, 1994) and in emotional self-regulation (Davidson & Irwin, 1999). It is suggested that the medial prefrontal cortex may be involved in the cognitive aspects, such as attention or appraisal, of emotional processing (Phan et al., 2002). A lesion study (Adolphs et al., 2000) demonstrated that the right somatosensory cortex is important for the

recognition of emotions from facial expressions, as lesions in this area were associated with impaired recognition of the six basic emotions (anger, disgust, fear, happiness, sadness and surprise). It was suggested that this is due to emotion recognition relying on internally simulating the somatosensory representations associated with a specific emotion. A further method for studying the neural correlates of emotions is pharmacological induction of emotions. Procaine selectively activates limbic structures, and procaine injections have been shown to lead to subjective emotional experiences accompanied by autonomic and endocrine changes, and increased bilateral blood flow in the anterior cingulate gyrus, the insula, and the amygdala and parahippocampal region (Servan-Schreiber & Perlstein, 1997; Servan-Schreiber et al., 1998), which supports the involvement of those neural regions in the processing of emotions.

Many studies have suggested that the amygdala plays a crucial role in the processing of fear (Calder et al., 2001; LeDoux, 2000; Phan et al., 2002). Some have widened this to detection of threat in the environment (Phillips et al., 1998b; Scott et al., 1997). However, amygdala activation has also been demonstrated in response to sad (Blair et al., 1999) and happy (Breiter et al., 1996) faces and vocalisations (Hamann & Mao, 2002; Sander & Scheich, 2001) and positive pictures (Hamann et al., 1999). The amygdala seems to be more sensitive to angry and fearful facial expressions than scenes (Hariri et al., 2002). Despite severe impairments of emotion, especially fear, recognition from facial (Adolphs et al., 1994) and auditory stimuli (Scott et al., 1997) amygdala lesions do not necessarily lead to an impairment in the recognition of facial expressions (Hamann & Adolphs, 1999) or emotional prosody (Adolphs & Tranel, 1999). These results indicate that the amygdala might not process fearful stimuli exclusively, but could have a more general role in detecting emotional importance or salience (Phan et al., 2002).

Perception of sad facial expressions has been associated with increased activity in the amygdala and in the temporal pole (Blair et al., 1999; Lee et al., 2002), but also with a pattern of non- specific brain activation (Kesler, 2001; Phillips et al., 1998a). Induction of sadness has been significantly linked with activation in the ventral anterior cingulate gyrus (Phan et al., 2002), although some studies report amygdala activation during sad mood induction (Schneider et al., 1997, 1998, 2000).

The basal ganglia have been associated with both perception of happy facial expressions and induction of happiness (Phan et al., 2002). However, some studies also reported activations in the left anterior cingulate and inferior frontal cortex (Dolan et al., 1996) and medial frontal cortex (Kesler, 2001) in response to positive emotional facial expressions, and amygdala in response to laughter (Sander & Scheich, 2001) and induction of happiness (Schneider et al., 1997).

Perception of angry facial expressions has been associated with activation in orbitofrontal and anterior cingulate cortex (Blair et al., 1999; Phan et al., 2002), the right posterior cingulate cortex and left medial frontal gyrus (Sprengelmeyer et al., 1998), and a network encompassing visual and frontal cortices (Kesler, 2001). However, there is no brain region which has consistently been associated with the perception of anger. Patients with lesions in the ventromedial prefrontal cortex were impaired in the re-experience of happiness, sadness, and to a certain degree fear, but were able to re-experience anger (Bechara et al., 2000). However, disruption of processing in the medial prefrontal cortex using transcranial magnetic stimulation suggests that this region is involved in the perception of anger, as it produced longer reaction times in response to angry faces, but not in response to happy faces (Harmer et al., 2001).

The review by Phan et al. (2002) also found an association of the basal ganglia with the processing of disgust, which is consistent with studies of patients with Huntington's disease and obsessive-compulsive disorder (Gray et al., 1997; Sprengelmeyer et al., 1996, 1997). However, on the basis of lesion studies (Adolphs et al., 2003; Calder et al., 2000) and functional neuroimaging studies (Berthoz et al., 2002; Phillips et al., 1997, 1998b; Schienle et al., 2002; Sprengelmeyer et al., 1998) it is also thought that the anterior insula is involved in the processing of disgusting stimuli. Another neuroimaging study reported increased insula activation during recall of events associated with the experience of guilt (Shin et al., 2000), which has been described as disgust directed towards the self (Power & Dalgleish, 1997). However, one study (Schienle et al., 2002) found anterior insula activation also in response to fearful pictures and therefore concluded that the anterior insula was not specifically involved in the processing of disgust. Nevertheless, the majority of evidence points to the insula playing a more important role in the perception of disgust

than in the perception of any other emotion. Due to the insula's connections (reviewed in section 1.3.4) and its proposed role in monitoring the internal environment (Damasio, 1994) it is not surprising to find it has also been associated with emotion induction by recall or imagery (Phan et al., 2002). Most studies to date have employed visual stimuli of disgust, and there is conflicting evidence regarding perception of disgust in other sensory modalities. A case study of a patient with a lesion of the insula and the striatum found impaired recognition of auditory stimuli of disgust (Calder et al., 2000). However, the only neuroimaging study using non-verbal vocal stimuli of disgust did not report any insula activation (Phillips et al., 1998b).

Most previous studies have investigated the neural correlates of one or two emotions, usually in one sensory modality (although there are some exceptions, for example (Kesler, 2001; Phillips et al., 1998b; Royet et al., 2000). This makes it difficult to directly compare results due to different methods in the selection of stimuli, image acquisition and analysis. In this chapter, therefore, the neural correlates underlying the perception of four emotions (anger, disgust, fear and sadness) in two sensory modalities (visual and auditory) are investigated.

3.2 Methods

3.2.1 Subjects

In order to keep the subject group as homogenous as possible only right-handed, male subjects participated in the experiment. Gender differences have been shown in neuroimaging studies of emotion (George et al., 1996; Kesler, 2001; Killgore & Yurgelun-Todd, 2001; Lee et al., 2002; Schneider et al., 2000).

Right-handedness was established using the Edinburgh Handedness Inventory (EHI). The EHI requires subjects to demonstrate 10 unimanual tasks and to state the degree of preference for the hand used as either strong (2 points) or weak (1 point). Assessments were completed once only for each subject and a handedness laterality quotient was calculated by subtracting the score for the left hand from the score for the right hand, dividing by the sum of both, and multiplying by 100. Only right-handed subjects (defined

as those scoring between +30 and +100 on the EHI; (Oldfield, 1971)) were recruited for the present study.

Premorbid IQ was assessed using the National Adult Reading Test – Revised (NART-R, (Nelson & Willison, 1991)). The NART requires a participant to read fifty irregular words, which do not follow the normal pronunciation rules. In order to successfully complete these reading tests, subjects must be familiar with the words. Premorbid IQ was estimated from the total pronunciation errors in this task. From these error scores an estimated WAIS IQ score was obtained using conversion charts.

Eighteen right-handed, male volunteers (mean age 28 years, mean NART IQ estimate 120, mean time spent in full-time education since the age of 5: 18 years) participated in four experiments in the same testing session. Exclusion criteria included history of brain injury and past and current psychiatric illness. Informed written consent was obtained from all subjects.

3.2.2 Experimental design

There were eight 5-minute experiments in total. Each experiment had a similar design, comprising 5 cycles of periodic alternation between 30s epochs of emotional stimuli (ON) and neutral stimuli (OFF).

In four experiments subjects were presented with images depicting facial expressions of anger, disgust, fear or sadness, contrasted with a neutral baseline expression. In the other four experiments subjects listened to non-verbal vocal expressions of anger, disgust, fear or sadness, also contrasted with a neutral baseline condition. Each subject participated in four out of the eight experiments, in which stimuli of two emotions were presented in two sensory modalities (table 3.2.2). The combinations of the different emotions were counterbalanced across the subjects, and each of the eight experiments was performed by 9 subjects. The order of presentation of stimuli within each condition, the first presentation (ON or OFF) for each experiment, and the presentation order of all experiments were counterbalanced across subjects.

During the presentation of sounds, and during the inter-stimulus interval (ISI) in the experiments with visual stimuli, the subjects viewed a central white cross on a black background.

The subjects were requested to decide the sex of each face or voice and press accordingly one of two buttons with their right thumb. The subjects were not informed that the purpose of this study was to examine the neural response to emotional stimuli. Previous studies have demonstrated that neural responses to emotional stimuli are dependent upon the nature of the task performed during viewing of the stimuli, and that performance of explicit, emotion labelling tasks is associated with reduced limbic and increased prefrontal activation (Hariri et al., 2000; Critchley et al., 2000; Lange et al., 2003).

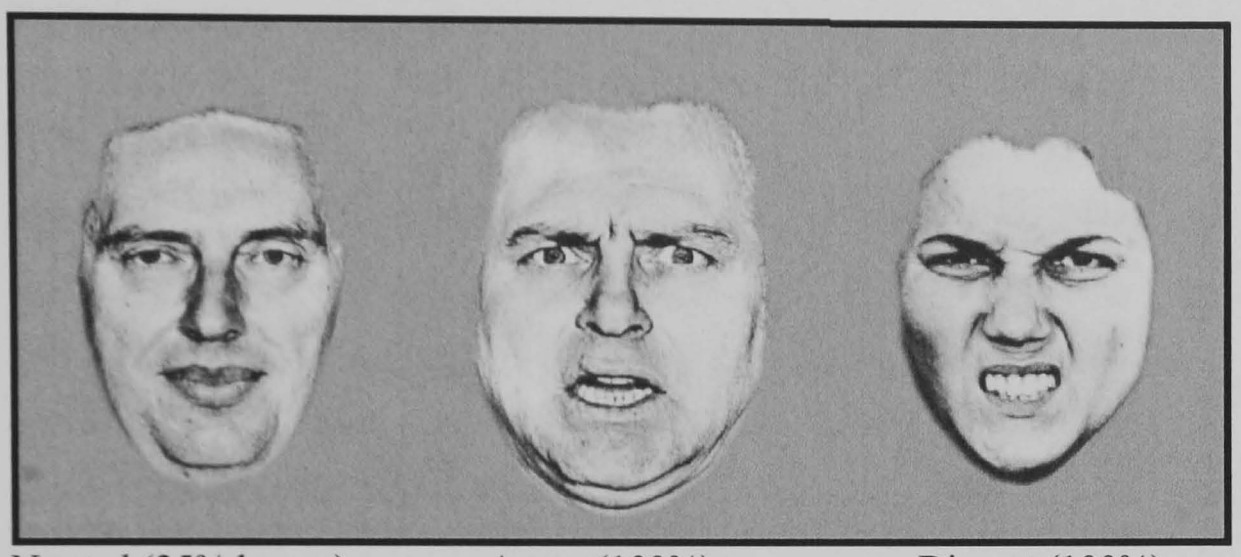
The sex-decision task employed in this thesis has been used in previous studies investigating the neural response to emotional stimuli (Morris et al., 1996; Phillips et al., 1997) and allows an identical task and response across the four experiments. This control condition also ensures more constraints on processing of stimuli than a passive control task.

After subjects had completed the scan they were given questionnaires to rate all stimuli used in all eight experiments. The questionnaires included a choice of category of emotion for each stimulus (anger, disgust, fear, happiness, neutral, sadness and surprise).

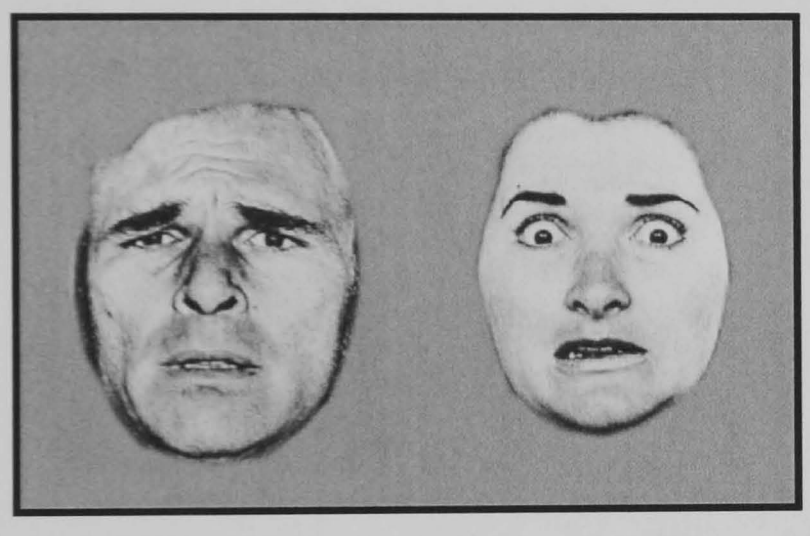
Table 3.2.2 Illustration of overlapping design.
For each subject and 5-minute experiment this table shows the emotion and the sensory modality it was presented in for all the subjects.

Subject	Experiment 1	Experiment 2	Experiment 3	Experiment 4
1	Fear, visual	Sadness, auditory	Fear, auditory	Sadness, visual
2	Sadness, auditory	Sadness, visual	Fear, visual	Fear, auditory
3	Sadness, visual	Fear, visual	Fear, auditory	Sadness, auditory
4	Anger, visual	Fear, auditory	Fear, visual	Anger, auditory
5	Fear, auditory	Anger, auditory	Anger, visual	Fear, visual
6	Fear, visual	Anger, auditory	Anger, visual	Fear, auditory
7	Disgust, visual	Fear, visual	Fear, auditory	Disgust, auditory
8	Fear, auditory	Fear, visual	Disgust, auditory	Disgust, visual
9	Disgust, visual	Fear, auditory	Disgust, auditory	Fear, visual
10	Sadness, visual	Anger, visual	Sadness, auditory	Anger, auditory
11	Anger, auditory	Sadness, visual	Anger, visual	Sadness, auditory
12	Sadness, auditory	Sadness, visual	Anger, auditory	Anger, visual
13	Disgust, auditory	Sadness, auditory	Sadness, visual	Disgust, visual
14	Disgust, visual	Disgust, auditory	Sadness, auditory	Sadness, visual
15	Sadness, auditory	Disgust, auditory	Disgust, visual	Sadness, visual
16	Disgust, auditory	Disgust, visual	Anger, auditory	Anger, visual
17	Anger, visual	Disgust, auditory	Disgust, visual	Anger, auditory
18	Anger, auditory	Anger, visual	Disgust, visual	Disgust, auditory

Figure 3.2.3a: Examples of the facial stimuli used in this study.



Neutral (25% happy) Anger (100%) Disgust (100%)



Sadness (100%) Fear (100%)

3.2.3 Stimuli

The stimuli depicting black-and-white grey scale images of prototypical facial expressions of anger, disgust, fear, sadness or neutral were taken from a standard series (Ekman and Friesen, 1976) of pictures of facial expressions of emotion containing male and female faces. These can be computer-transformed to create different intensities of each facial expression (Calder et al., 1997). The pictures showing facial expressions of the four emotions were all displayed at an intensity of 100% (figure 3.2.3a). A mildly happy expression (75% neutral, 25% happy) was used as the neutral baseline, the 100% neutral facial expressions (all facial muscles relaxed) from this series can appear cold and threatening, because of the social convention to signal approval in normal interactions.

During each ON or OFF epoch eight different standard faces (identities) from the Ekman-Friesen series displaying an emotion (anger, disgust, fear or sadness) or neutral facial expression were each presented for 3s with an inter-stimulus interval (ISI) of 0.75s (figure 3.2.3b).

Non-verbal sounds displaying basic emotions including anger, disgust, fear, sadness and mild happiness were employed (Scott et al., 1997). These vocalizations were produced by two native English speakers (one male and one female), and have been used in several previous studies (Scott et al., 1997; Phillips et al., 1998; Morris et al., 1999). During each epoch (ON or OFF) eight different sounds expressing an emotion (anger, disgust, fear or sadness) or mild happiness (mild happiness was used as a neutral baseline to match the mildly happy facial expressions used) were each presented for 3s, with an interstimulus interval of 0.75s, using the same software as for the presentation of the facial stimuli.

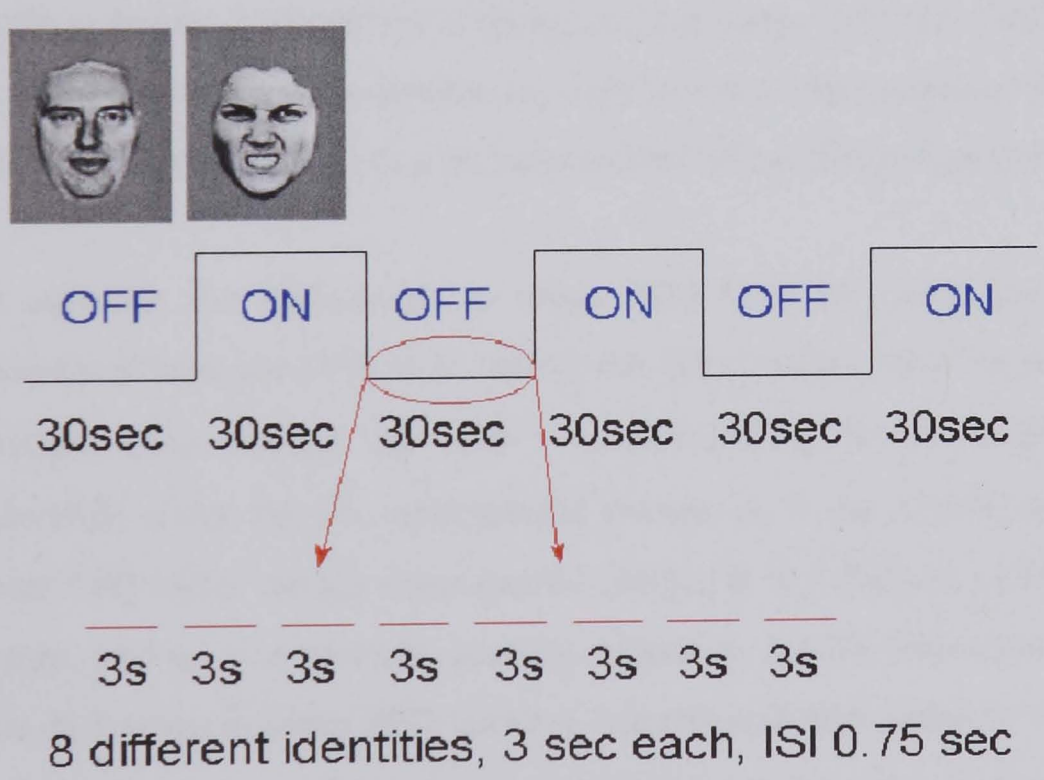


Figure 3.2.3b: Illustration of the experimental design.
This figure shows alternating 30s ON/OFF epochs. During each 30s epoch 8 different identities displaying either neutral (OFF) or emotional (ON) faces are shown for 3s each with an inter-stimulus interval of 0.75s.

3.2.4 Image Acquisition

The image acquisition was performed as described in detail in section 2.2.

3.2.5 Analysis

The data were analysed as described in section 2.4.1. Following generic brain activation mapping the data were compared within emotion and between modality to investigate whether similar neural circuits were activated in response to the same emotion presented in different sensory modalities. Voxel-wise comparisons between group activation can then be made by random permutation of the data at each voxel between groups and calculating the probability of a group difference under the null hypothesis from the rate of occurrence, on permutation, of group differences as large as that in the original data (Edgington, 1995). This is in fact the gold-standard method against which parametric methods should be compared. As the data are individually standardised (for each subject) against the error variance before construction of group maps in Talairach space and as the subsequent testing by permutation does not assume any distributional approximation for the data at each voxel, this method also represents a de facto method of accommodating random effects.

To estimate the differences in mean FPQ between conditions the repeated measures analysis of variance (ANOVA) model was fitted at each voxel of the observed FPQ maps in standard space: $FPQ_{i,j} = \beta_0 + \beta_j \mathbf{E} + \mathbf{e}_{i,j}$. Here, $FPQ_{i,j}$ denotes standardised power in the ith individual under the jth experimental design, β_0 is the overall mean FPQ, $\beta_0 + \beta_j$ is the mean FPQ under the jth experimental design, $\mathbf{E}$ is a dummy variable coding experimental design, and $\mathbf{e}_{i,j}$ is a residual quantity unique to the ith individual. The null hypothesis of zero difference in mean FPQ between experiments was tested by comparing the coefficient β_j to critical values of its nonparametrically ascertained null distribution. To do this, the elements of $\mathbf{E}$ were randomly permuted 10 times at each voxel, β_j was estimated at each voxel after each permutation, and these estimates were pooled over all intracerebral voxels to sample the permutation distribution of β_j (Bullmore et al., 1999). For a two-tailed test of size $p=0.05$, the critical values were the 100 x p/2th and 100 x (1-p/2)th percentile values of

the permutation distribution. Differences in mean FPQ between conditions were tested for significance only at those voxels which were generically activated by one or both of the conditions considered independently, thereby substantially reducing the search volume or number of tests conducted.

3.3 Results

3.3.1 Behavioural Results

3.3.1.1 Sex Decision Task

During the fMRI scan subjects were asked to indicate whether the face or voice presented was male or female. For technical reasons response files were only collected for 15 of the 18 subjects, for the other three subjects the computer failed to record the responses. The results are summarised in table 3.3.1.1.

Table 3.3.1.1: Percent of correct answers during the sex identification task.

Sensory Modality	Anger	Disgust	Fear	Sadness	Total
Vision	94.8	96.8	94.8	97.9	96.1
Audition	65.4	64.2	79.9	75.3	71.2

Performance did not decline during the experiments, which indicates that subjects were paying attention to all stimuli presented. As can be seen from the table there is a significant difference between the number of stimuli that were correctly identified as male or female in the two sensory modalities, with non-verbal vocal stimuli being more ambiguous.

3.3.1.2 Post-scan Rating of Stimuli

The results of the post-scan rating are summarized in table 3.3.1.2 below.

Table 3.3.1.2: Results of post-scan questionnaires.
Results are given in percent correctly identified stimuli, (range) indicates the range of percent correctly identified individual stimuli within each emotional category.

Sensory Modality	Anger	Disgust	Fear	Neutral	Sadness
Vision	69.5 (16.7-100)	84 (61.1-100)	77.1 (50-100)	98.6 (94.4-100)	74.3 (50-100)
Audition	70.8 (44.4-88.9)	98.6 (94.4-100)	79.2 (55.6-94.4)	71.5 (22.1-94.4)	95.1 (66.7-100)
Total	70.1	91.3	78.1	85.1	84.7

Overall the disgusting stimuli were most often identified correctly, with anger being the emotion most difficult to identify. Anger and fear were identified correctly at similar percentages in both sensory modalities, whereas disgust and sadness were correctly identified to a higher degree in the auditory modality and neutral stimuli were correctly identified more easily in the visual modality. As can be seen from the ranges, most categories have stimuli that are always identified correctly (100%) and other stimuli that are more ambiguous. Although the Ekman & Friesen faces have been used in many previous experiments they could clearly be improved to ensure a more homogeneous group of stimuli that are identified more easily.

Some patterns of mis-identification emerged. Angry faces were most commonly mistaken for disgusted faces (13.2%), whereas angry voices were most commonly mistaken for neutral ones (15.3%) with disgusting ones (8.3%) taking second place. Conversely, facial expressions of disgust were most commonly mistaken for expressions of anger (11.1%). Fearful visual and auditory stimuli were most commonly mistaken for displaying surprise (17.4% and 11.1%, respectively), similarly neutral voices were often thought to display surprise (26.4%).

Individual subjects' ratings ranged from 52.5% to 95% correct for the visual stimuli and from 65%-92.5% correct for the auditory stimuli, giving means of 81.3% and 82.9% respectively.

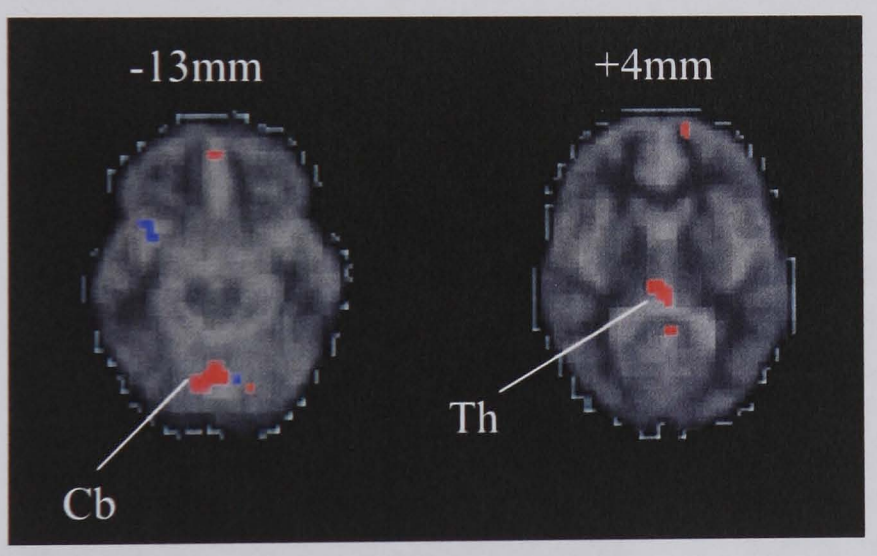
3.3.2 Generic Brain Activation

Major regions of generic brain activation in response to visual presentations of anger

Generic brain activation was demonstrated in the right thalamus, the left precuneus (BA 7), the right inferior temporal gyrus (BA 20) and bilateral cerebellum (see table 3.3.2.Ia and figure 3.3.2.Ia).

Major regions of generic brain activation in response to auditory presentations of anger

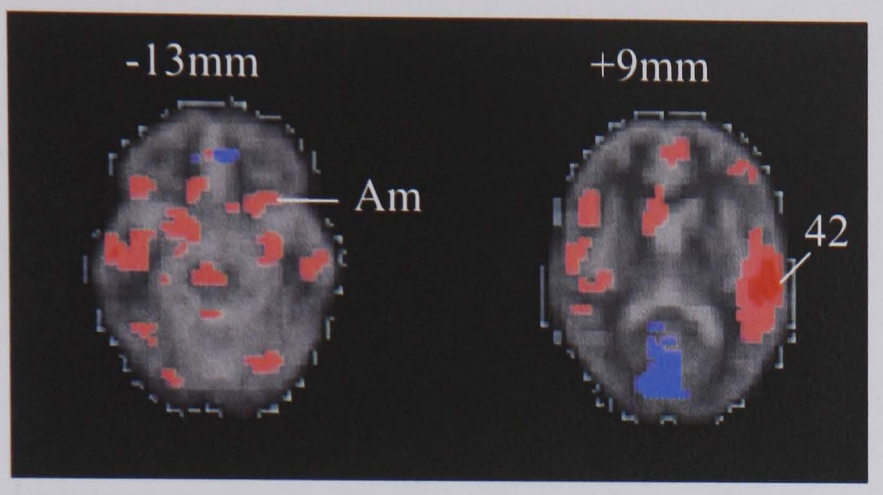
There was significantly more activation in response to angry auditory stimuli than to angry visual stimuli. A wide network of frontal, temporal and parietal brain regions was activated. The areas included bilateral auditory cortex, right dorsal and ventral anterior cingulate gyrus, bilateral parahippocampal gyrus and amygdala, bilateral inferior frontal gyrus and right putamen. Other temporal, frontal and parietal areas of activation are detailed in table 3.3.2.Ib and figure 3.3.2Ib.



Cb = cerebellum, Th = Thalamus

Figure 3.3.2.Ia: Brain activation in response to facial expressions of anger

The numbers above the transverse sections indicate the distance in mm from the trans-callosal line. The right side of the brain is shown on the left side of each panel and vice versa. Voxels activated at $p<0.004$ by facial expressions of anger are shown in red, and voxels activated during the neutral condition are shown in blue.



Am = Amygdala, 42 = BA42, auditory cortex

Figure 3.3.2.Ib: Brain activation in response to vocal expressions of anger.
The numbers above the transverse sections indicate the distance in mm from the transcallosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by vocal expressions of anger are shown in red, and voxels activated during the neutral condition are shown in blue.

Major regions of generic brain activation in response to visual presentations of disgust

The only significantly activated voxels in response to facial expressions of disgust were found in right supramarginal gyrus and inferior parietal lobule and in the left middle frontal gyrus (BA 46) (table 3.3.2.IIa and figure 3.3.2.IIa).

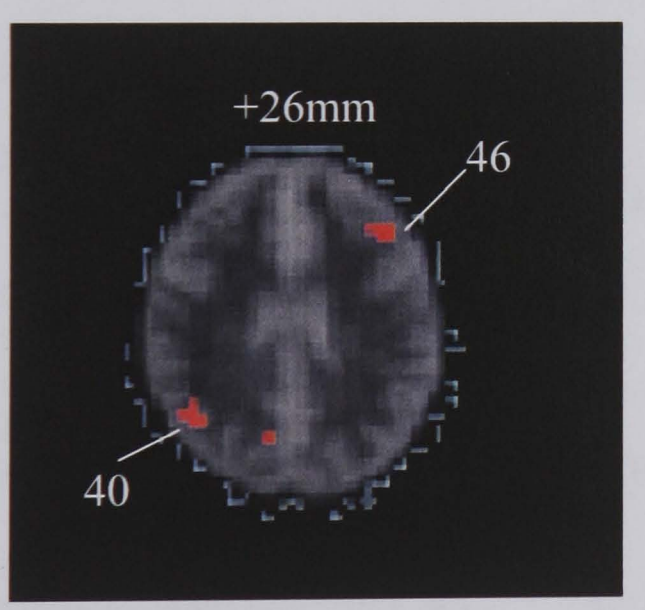


Figure 3.3.2.IIa: Brain activation in response to facial expressions of disgust
The numbers above the transverse sections indicate the distance in mm from the transcallosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by facial expressions of disgust are shown in red.

40 = BA40, inferior parietal cortex, 46 = BA46, dorsolateral prefrontal cortex

Table 3.3.2 I a) Facial expressions of anger and b) vocal expressions of anger: main generically activated brain areas

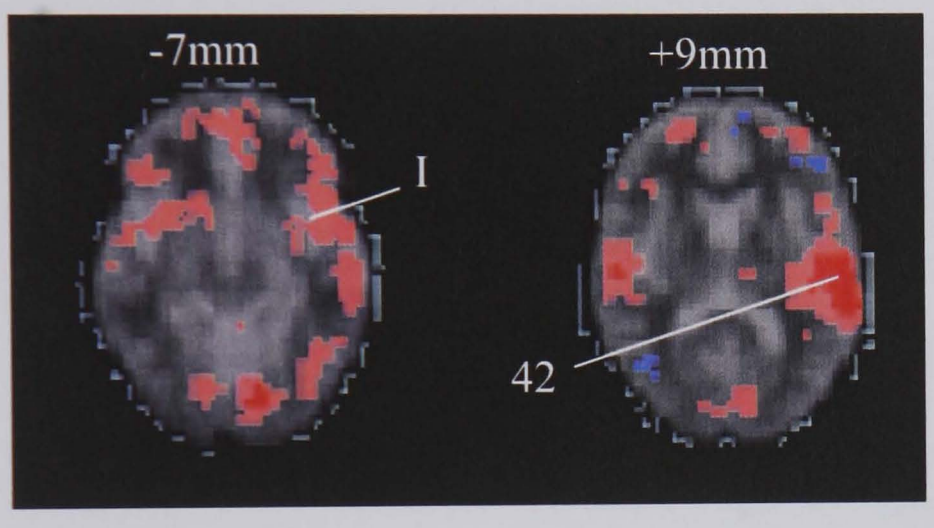
Region activated (approximate Brodman area)	Side	x[a]	y[a]	z[a]	Number of voxels[b]
a) Facial expressions					
Cerebellum	L/R	0	-67	-13	13
Thalamus	R	4	-30	4	8
Precuneus (BA 17)	L	-7	-60	37	7
Inferior temporal gyrus (BA 20)	L	-43	-13	-29	5
b) Vocal expressions					
Superior temporal gyrus (BA22/42)	L/R	-50	-30	9	84
		40	-30	15	14
Anterior cingulate gyrus (BA24/25)	R	7	17	-18	78
		4	-17	42	17
Heschl gyrus (BA 41)	L	-43	-23	15	57
Parahippocampal gyrus (BA 35)	L/R	28	-23	-18	43
		-21	-17	-24	14
Medial frontal gyrus (BA 8/9)	R	4	26	42	34
Inferior frontal gyrus (BA 44/47)	L/R	-47	4	20	29
		43	10	26	27
Middle temporal gyrus (BA 21)	L/R	53	-17	-13	28
		-50	-30	-7	12
Inferior temporal gyrus (BA 37)	L	-43	-56	-2	24
Amygdala	L/R	-28	-10	-7	22
		17	-4	-13	21
Cerebellum	L	-21	-56	-18	20
Putamen	R	11	0	4	18
Caudate nucleus	R	11	4	9	16
Inferior parietal lobule (BA 40)	L	-50	-23	26	16
Precentral gyrus (BA 6)	L/R	-43	0	31	15
		43	0	37	10
Retrosplenial gyrus (BA 29)	L	-11	-33	20	15
Supramarginal gyrus (BA 40)	L	-36	-50	37	12

[a] Talairach coordinates refer to the voxel with the maximum FPQ value in each regional cluster.

[b] All activated voxels were identified by a one-tailed t-test of the null hypothesis that median FPQ is not determined by experimental design. The probability threshold for activation was $p \leq 0.004$.

Major regions of generic brain activation in response to auditory presentations of disgust

As was found for angry stimuli, there was significantly more activation in response to auditory stimuli of disgust than there was in response to visual stimuli. The main regions of generic brain activation in response to auditory stimuli of disgust included bilateral auditory cortex (BA 42), bilateral middle temporal gyrus (BA 21), dorsal and ventral anterior cingulate gyrus (BA 24 and 32), bilateral cerebellum, medial frontal gyrus and inferior frontal gyrus. Activation was also demonstrated in the left anterior insula (table 3.3.2.IIb and figure3.3.2.IIb).



I = Insula, 42 = BA42, auditory cortex

Figure 3.3.2.IIb: Brain activation in response to vocal expressions of disgust.
The numbers above the transverse sections indicate the distance in mm from the transcallosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by vocal expressions of disgust are shown in red.

Table 3.3.2.II a) Facial expressions of disgust and b) vocal expressions of disgust: main generically activated brain areas

Region activated (approximate Brodman area)	Side	x[a]	y[a]	z[a]	Number of activated voxels[b]
a) Facial expressions					
Supramarginal gyrus (BA 39)	R	43	-56	31	9
Middle frontal gyrus (BA 46)	L	-36	20	26	5
Inferior parietal lobule (BA 40)	R	43	-52	26	5
b) Vocal expressions					
Superior temporal gyrus (BA22/42)	L/R	-50	-20	4	125
		53	-23	9	33
Lingual gyrus (BA 18)	L/R	-11	-80	-2	93
		15	-76	-7	15
Insula	L	-40	7	-7	56
Middle temporal gyrus (BA 21)	L/R	53	-17	-2	47
		-53	-30	-7	21
Anterior cingulate gyrus (BA24/32)	R	4	13	31	43
		4	39	-7	32
Cerebellum	L/R	-28	-73	-24	42
		7	-60	-24	28
Inferior parietal lobule (BA 40)	L	-50	-37	26	36
Cuneus (BA 17)	L	-7	-80	9	34
Medial frontal gyrus (BA 32)	L/R	7	17	37	30
		-7	26	-13	27
Gyrus rectus (BA 11)	R	7	30	-18	22
Inferior frontal gyrus (BA 45)	L/R	-40	20	20	19
		43	20	-7	12
Precentral gyrus (BA 6)	L	-40	-10	42	14
Corpus callosum	R	4	17	20	10

[a] Talairach coordinates refer to the voxel with the maximum FPQ value in each regional cluster.

[b] All activated voxels were identified by a one-tailed t-test of the null hypothesis that median FPQ is not determined by experimental design. The probability threshold for activation was $p \leq 0.004$.

Table 3.3.2.III a) Facial expressions of fear and b) vocal expressions of fear: main generically activated brain areas

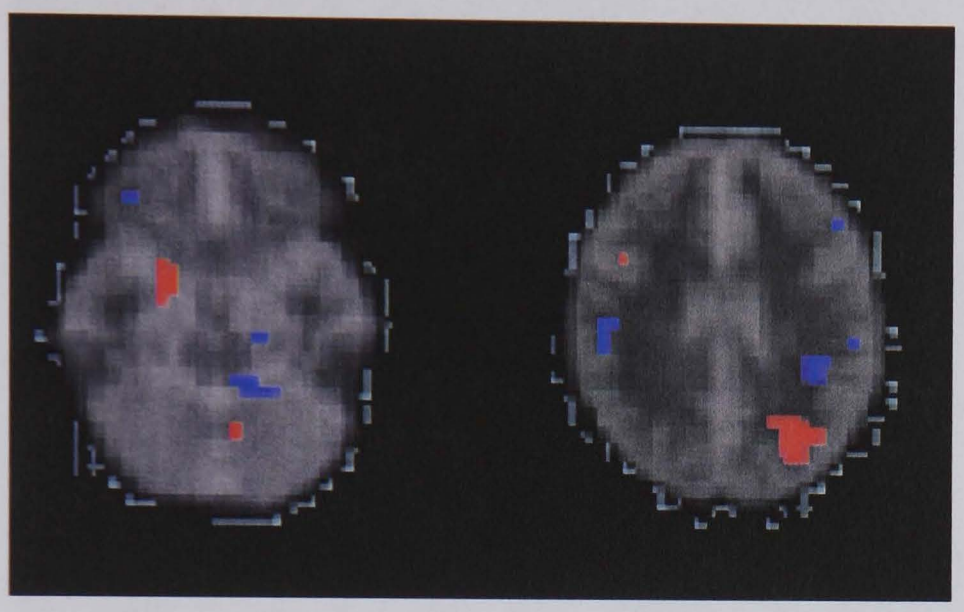
Region activated (approximate Brodman area)	Side	x[a]	y[a]	z[a]	Number of activated voxels[b]
a) Facial expressions					
Precuneus (BA 31)	L	-25	-60	26	19
Retrosplenial cortex (BA 27)	R	11	-30	-2	17
Superior occipital gyrus (BA 19)	L	-28	-67	31	14
Amygdala	R	21	-7	-18	8
Supramarginal Gyrus (BA 40)	R	50	-30	31	5
b) Vocal expressions					
Posterior cingulate gyrus (BA 23/31)	L/R	4	-52	37	122
		7	-50	15	60
		-7	-50	26	58
Middle occipital gyrus (BA 19)	R	40	-69	-7	59
Retrosplenial cortex (BA 30)	R	7	-52	9	52
Anterior cingulate gyrus (BA 32)	L	-4	39	-2	43
Precuneus (BA 31)	R	25	-69	20	38
Cerebellum	L/R	-17	-76	-18	28
		21	-60	-13	13
Medial frontal gyrus (BA 9/10/32)	L/R	4	46	-7	26
		-17	37	26	24
		-11	43	20	17
Middle frontal gyrus (BA 9)	L	-32	7	37	21
Lingual gyrus (BA 18)	R	4	-80	4	14
Supramarginal gyrus (BA 40)	L/R	36	-46	37	14
		-43	46	37	13
Orbitofrontal cortex (BA 11)	L	-4	46	-13	12
Putamen	R	4	0	4	11
Superior temporal gyrus (BA 22)	L	-43	-50	20	11

[a] Talairach coordinates refer to the voxel with the maximum FPQ value in each regional cluster.

[b] All activated voxels were identified by a one-tailed t-test of the null hypothesis that median FPQ is not determined by experimental design. The probability threshold for activation was $p \leq 0.004$.

Major regions of generic brain activation in response to visual presentations of fear

Facial expressions of fear activated left-sided retrosplenial cortex (BA 31), right amygdala, and right supramarginal gyrus (BA 40) (table 3.3.2.IIIa and figure 3.3.2.IIIa).

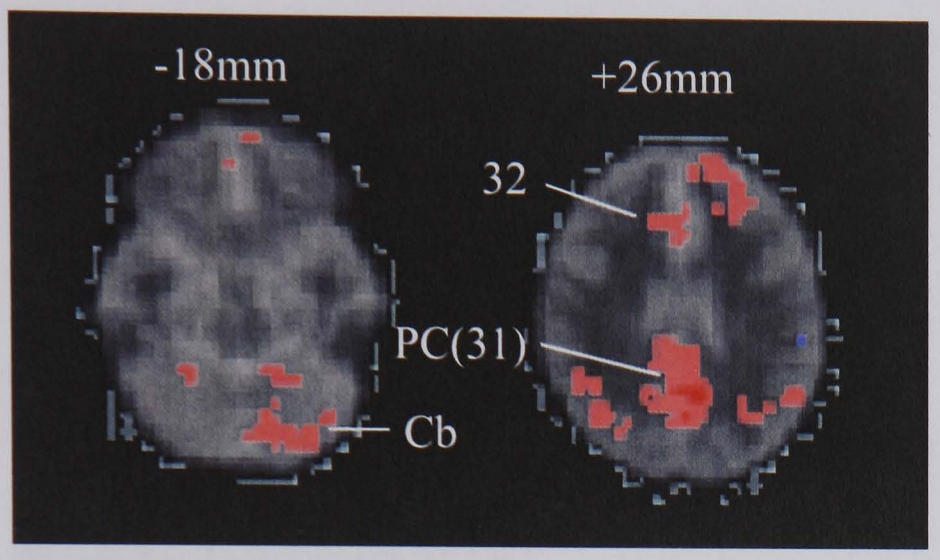


Am = Amygdala, 31 = BA31, retorsplenial cortex

Figure 3.3.2.IIIa: Brain activation in response to facial expressions of fear.
The numbers above the transverse sections indicate the distance in mm from the trans-callosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by facial expressions of fear are shown in red, and voxels activated during the neutral condition are shown in blue.

Major regions of generic brain activation in response to auditory presentations of fear

As noted for angry and disgusting auditory stimuli, there was more generic brain activation in response to fearful auditory stimuli than there was in response to fearful visual stimuli. The areas of activation in response to fearful auditory stimuli include bilateral posterior cingulate gyrus (BA 23 and 31), left anterior cingulate gyrus (BA 32), bilateral cerebellum, bilateral medial frontal gyrus both dorsal and ventral, right putamen, and left superior temporal gyrus (BA 22) (table 3.3.2.IIIb and figure 3.3.2.IIIb).

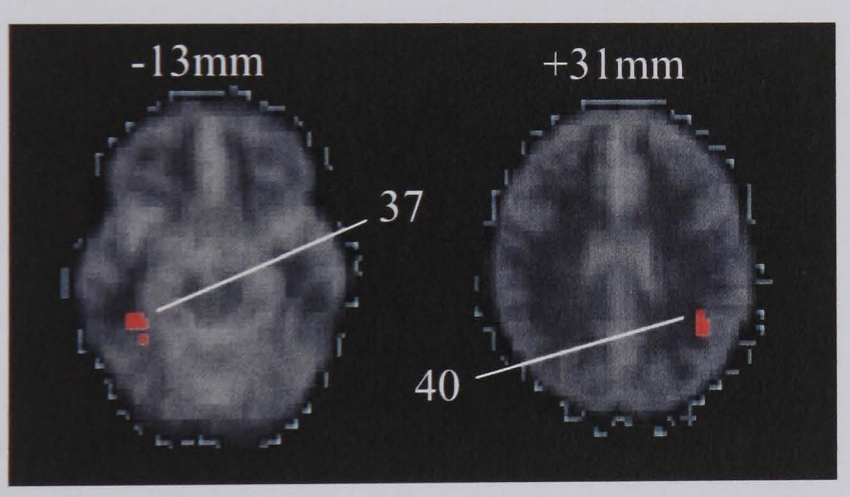


Cb = Cerebellum, 32 = BA32, anterior cingulate cortex, PC(31) = BA31, posterior cingulate gyrus

Figure 3.3.2.IIIb: Brain activation in response to vocal expressions of fear.
The numbers above the transverse sections indicate the distance in mm from the trans-callosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by vocal expressions of fear are shown in red.

Major regions of generic brain activation in response to visual presentations of sadness

The main regions of generic brain activation in response to facial expression of sadness were left supramarginal gyrus and cerebellum, and right fusiform gyrus (table 3.3.2.IVa and figure 3.3.2.IVa).



37 = BA37, fusiform gyrus, 40 = BA40, supramarginal gyrus

Figure 3.3.2.IVa: Brain activation in response to facial expressions of sadness.
The numbers above the transverse sections indicate the distance in mm from the trans-callosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by facial expressions of sadness are shown in red.

Major regions of generic brain activation in response to auditory presentations of sadness

In response to auditory stimuli of sadness brain activation was demonstrated in bilateral medial frontal lobe, bilateral auditory cortex (BA 42) and middle temporal gyrus (BA 21), right cerebellum, and right posterior insula. Additional areas of activation are listed in table 3.3.2.IVb and figure 3.3.2.IVb.

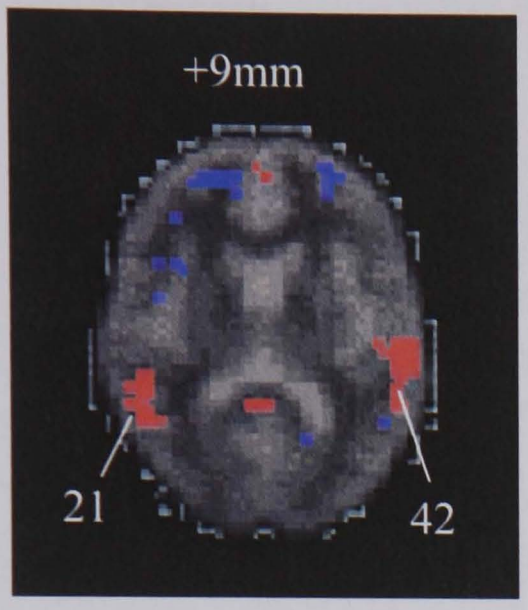


Figure 3.3.2.IVb: Brain activation in response to vocal expressions of sadness.
The numbers above the transverse sections indicate the distance in mm from the trans-callosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by vocal expressions of sadness are shown in red, and voxels activated during the neutral condition are shown in blue.

21 = BA21, middle temporal gyrus, 42 = BA42, auditory cortex

Table 3.3.1.3.IV a) Facial expressions of sadness and b) vocal expressions of sadness: main generically activated brain areas

Region activated (approximate Brodman area)	Side	x[a]	y[a]	z[a]	Number of activated voxels[b]
a) Facial expressions					
Supramarginal gyrus (BA 40)	L	-36	-46	26	5
Fusiform gyrus (BA 37)	R	36	-43	-13	5
Cerebellum	L	-28	-43	-18	5
b) Vocal expressions					
Medial frontal lobe (BA 32)	L/R	-25	26	26	24
		4	37	20	15
Superior temporal gyrus (BA 22/42)	L/R	-53	-26	9	19
		47	-46	15	16
Posterior insula	R	43	-10	4	19
Cerebellum	R	11	-73	-7	18
Middle temporal gyrus (BA 21)	L/R	-57	-20	-7	17
		50	-50	9	16
Precuneus (BA 31)	R	0	-69	20	10
Anterior temporal pole (BA 38)	R	28	4	-24	10

[a] Talairach coordinates refer to the voxel with the maximum FPQ value in each regional cluster.
[b] All activated voxels were identified by a one-tailed t-test of the null hypothesis that median FPQ is not determined by experimental design. The probability threshold for activation was $p \leq 0.004$.

3.3.3 Summary

Across all emotions there was more significant brain activation in response to auditory stimuli than to visual stimuli. Most visual stimuli activated supramarginal gyrus (apart from facial expressions of anger). All auditory stimuli activated auditory cortex (BA 42) and the anterior cingulate gyrus. As expected the amygdala was only activated in response to facial expressions of fear. No insula activation was observed in response to facial expressions of disgust. Other areas commonly activated by emotional auditory stimuli include cerebellum, medial frontal gyrus, and middle temporal gyrus. Only angry vocal stimuli activated bilateral amygdalae; disgusting vocal stimuli activated the left insula.

3.3.4 Comparisons of generic brain activation between sensory modality within emotion

Comparison of brain activation in response to auditory and visual stimuli of anger

The only brain region that was activated significantly more in response to facial expressions of anger than in response to vocal expressions of anger was the cerebellum. Angry auditory stimuli caused greater activation in the superior temporal gyrus (BA 21 and BA 42), which is part of the auditory cortex, and in BA 6 when compared to the response to facial expressions of anger.

Comparison of brain activation in response to auditory and visual stimuli of disgust

When comparing auditory and visual stimuli of disgust, the inferior parietal lobule (BA 40) was activated to a greater extent in response to facial expressions of disgust whereas the auditory cortex (BA 22 and BA 42) and parts of primary visual cortex were activated to a greater extent in response to auditory stimuli of disgust.

Comparison of brain activation in response to auditory and visual stimuli of fear

Fearful faces elicited a greater response than fearful voices in the inferior temporal lobe (BA 35), whereas auditory stimuli of fear activated the posterior cingulate gyrus (BA 31) to a significantly greater extent than facial expressions of fear.

Comparison of brain activation in response to auditory and visual stimuli of sadness

The inferior parietal lobule was activated to a greater extent in response to facial than in response to vocal expressions of sadness. Sad vocal stimuli elicited a greater response than sad faces in the primary visual cortex (BA 18), inferior frontal gyrus (BA 44), Supramarginal gyrus (BA 40), medial frontal gyrus (BA 9) and anterior cingulate gyrus (BA 32).

Table 3.3.4 Within emotion, between sensory modality comparisons of generic brain activation

Region activated (approximate Brodman area)	Side	x[a]	y[a]	z[a]
a) Anger: Visual > Auditory				
Cerebellum	R	29	-61	-24
Auditory > Visual				
Superior temporal gyrus (BA42, BA21)	L/R	-49	-21	7
		53	-10	-5.5
BA6	R	0	-18	47
b) Disgust: Visual > Auditory				
Inferior parietal lobule (BA40)	R	43	-54	28
Auditory > Visual				
Primary visual cortex (BA18)	L	-2	-80	-3
Superior temporal gyrus (BA22, BA42)	R/L	53	-12	4
		-48	-19	7
c) Fear: Visual > Auditory				
Inferior temporal lobe (BA35)	L	-20	-13	1
Auditory > Visual				
Posterior cingulate gyrus (BA31)	R	5	-50	26
d) Sadness: Visual > Auditory				
Inferior parietal lobule (BA40)	L	-38	-45	26
Auditory > Visual				
Primary visual cortex (BA18)	R	9	-75	-7
Inferior frontal gyrus (BA44)	R	47	13	15
Supramarginal gyrus (BA40)	L	-49	-53	31
Medial frontal gyrus (BA9)	R	39	18	34
Anterior cingulate gyrus (BA32)	R	4	19	40

3.4 Discussion

In this chapter the neural responses to visual and auditory stimuli of anger, disgust, fear and sadness were investigated. Overall, generic brain activation was much more pronounced in response to auditory stimuli than to visual stimuli. This increased activation could be due to the auditory stimuli requiring extra attention, as the MRI scanner is quite noisy. Volunteers wear headphones to dampen scanner noise, but the percentage of correct results for the sex decision task is much lower for the auditory stimuli than it is for the visual stimuli (table 3.3.1.1). As the subjects were not asked to identify the sex of the voice/face in the after-scan questionnaire but only the emotion it is impossible to determine whether the auditory stimuli are inherently more ambiguous as far as their identity is concerned or whether the scanner noise made it more difficult to correctly identify the sex of the auditory stimuli. In addition, the visual stimuli are static in nature compared with the dynamic nature of the auditory stimuli, which may also have contributed to the auditory stimuli demanding more attention. However, regardless of the cause of the ambiguity the auditory stimuli were clearly more difficult to identify correctly than the visual stimuli. All auditory conditions activated the superior temporal gyrus in the region of the auditory cortex (Brodmann areas (BA) 22 and 42) and the anterior cingulate gyrus (BA 24 and 32). Auditory cortex activation was unexpected as both the emotional and the control condition consisted of auditory stimuli. As the auditory stimuli were more difficult to identify than the visual stimuli, this could have led to an increased attentional demand during processing of the auditory stimuli, which could explain activation of the anterior cingulate cortex and auditory cortex in response to the auditory stimuli. The anterior cingulate gyrus is thought to be involved in attentional processes such as error monitoring (Carter et al., 1998), pain (Davis et al., 1997), but also during attention to emotional stimuli and emotional awareness (Lane et al., 1997a, 1998). Previous functional neuroimaging studies have reported increased sensory cortex activation in response to attention to emotional stimuli. This has been shown for emotional visual stimuli in response to which there was increased activation in the visual cortex (Buechel & Friston, 1997; Morris et al., 1998); increased activation has also been reported in somatosensory regions of the parietal cortex in response to tactile attentional tasks (Burton et al., 1999); and attention has been shown to

modulate primary and secondary auditory cortex activation in response to auditory stimuli (Jaencke et al., 1999).

In response to the auditory stimuli there was also some activation in visual areas such as BA 18 and 19. This has also been reported in previous studies (Phillips et al., 1998b). Visual imagery during presentation of emotional auditory stimuli could have caused activation of visual cortex.

There was increased activation in secondary visual cortex in all response to all visual stimuli. The stimuli were matched for everything except emotional context, and this finding repeats the pattern observed with auditory stimuli. Again, this might suggest that perception of emotional stimuli modulates activity in the sensory cortex of the modality in which the stimuli are presented. Similar modulation in response to presentation of emotional stimuli has been reported before (Buechel & Friston, 1997; Morris et al., 1998). The cerebellum was activated by most conditions. This was surprising, but many previous studies (Lane et al., 1997b; Morris et al., 1996, 1998; Schienle et al., 2002; Schneider et al., 2000) investigating the neural correlates of emotion also reported cerebellar activation, though usually without drawing attention to it in the discussion. One theory proposes the involvement of the cerebellum in emotion perception per se (Schmahmann & Sherman, 1997).

With regard to individual emotions, previous findings were only partly replicated. Activation of the amygdala was demonstrated in response to facial presentations of fear, which is in accordance with previous studies (Breiter et al., 1996; Morris et al., 1996, 1998; Phillips et al., 1998b). Vocal expressions of fear did not lead to increased activation in the amygdala, but instead in areas associated with emotion processing per se, such as the posterior cingulate gyrus, the medial prefrontal cortex, and the ventral anterior cingulate gyrus. However, vocal presentations of anger did lead to increased amygdala activation, possibly as they were threatening and the amygdala has also been associated with threat perception (Scott et al., 1997). Angry faces did not cause amygdala activation, which contradicts this explanation as angry faces are also threatening. Unlike previous studies there was no amygdala activation in response to sad stimuli (Blair et al., 1999; Lee et al.,

2002) of either modality, or in response to stimuli of disgust (Schienle et al., 2002). The results with regard to the amygdala are therefore difficult to interpret, as they do not seem to follow a consistent pattern.

Insula activation was demonstrated only in response to vocal presentations of disgust but not in response to facial expressions of disgust. The absence of insula activation in response to facial expressions of disgust was particularly surprising as this finding has been replicated several times (Schienle et al., 2002; Sprengelmeyer et al., 1998), with some studies using the same stimuli and experimental design as used in the experiment described here (Phillips et al., 1997, 1998b, 1999). Based on lesion studies (Calder et al., 2000) and a meta-analysis (Phan et al., 2002), activation in response to auditory and visual stimuli of disgust was also expected in the basal ganglia. However, this was not observed, contradicting previous findings.

According to the meta-analysis by Phan et al. (2002), processing of sadness has been associated with activation in the subcallosal gyrus. This was not observed in response to visual or auditory stimuli of sadness. Both sets of stimuli only led to increased activation in non-specific emotion processing areas, which has also been observed in a previous study (Phillips et al., 1998a). A previous study investigating the neural correlates of emotional vocalisations reported anterior insula and middle temporal gyrus activation in response to fearful, happy and sad vocalisations (Morris et al., 1999). In the experiment performed in this thesis middle temporal gyrus activation was observed in response to angry, disgusted and sad vocalisations, but not in response to fearful non-verbal vocal stimuli, again only partly replicating previous findings.

The within-emotion between sensory modality comparisons of the GBAMs also did not yield any consistent results. In summary, the results from this experiment fit only partially with previously observed brain activation in response to emotional stimuli. This could be due to the complex nature of the experiment, with each subject being presented with two different emotions in two sensory modalities, and different subjects being exposed to different combinations of emotions. It is possible that the presentation of the first emotion could influence the neural networks activated in response to the emotional stimuli presented

subsequently, so that the order of presentation or the context in which emotional stimuli are presented could influence the results. In order to investigate this possibility the data were re-analysed and new experiments were performed, which are described in chapter 4.

Chapter 4

Potential Order and Context Effects

4.1 Introduction

In functional neuroimaging studies it is often assumed that the neural responses to stimuli remain the same, irrespective of the order in which experiments are performed or of repetition of experiments. Many studies have used functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) to examine the neural responses to emotional stimuli (George et al., 1995; Lane et al., 1997b; Morris et al., 1996; Phillips et al., 1997, 1998b; Sprengelmeyer et al., 1998). Most of these studies have not paid attention to possible effects of repeated presentation of emotional stimuli. My findings from the previous chapter show that there are possible effects of order or context in which emotional stimuli are being presented. One approach to clarify the extent to which stimulus presentation factors affect the neural response to an emotional stimulus is to repeat a task and then to determine whether the response remains constant over the course of successive stimulus presentation, or to present other emotional stimuli before presenting the target emotion and testing if this has an effect on the neural systems activated in response to the target emotion.

Changes in amygdala activation over time in response to presentation of fearful facial stimuli have been reported in several studies (Breiter et al., 1996; Morris et al., 1998; Phillips et al., 2001; Wright et al., 2001). These show habituation within the amygdala, especially the right amygdala, to fearful faces within the time-span of one experiment, typically 5 minutes. There have been no studies looking at repeated presentations of fearful faces in several 5-minute experiments, and no study has explicitly investigated effects of preceding presentation of fearful facial expressions with presentations of different emotions. There have been no studies investigating potential changes in the insular response to repeated presentation of facial expressions of disgust or to presentations of facial expressions of disgust preceded by other emotions.

In the preceding chapter findings from previous studies were only partly replicated. Activation of the amygdala was demonstrated in response to facial presentations of fear and vocal presentations of anger, and insula activation was demonstrated in response to vocal presentations of disgust but not in response to facial expressions of disgust. The absence of insula activation in response to facial expressions of disgust was particularly surprising as this finding has been replicated several times using different experimental designs (Sprengelmeyer et al., 1998; Surguladze et al., 2003) but also using the same stimuli and block design as used in this thesis (Phillips et al., 1997, 1998b, 2000). It is possible that this discrepancy was due to the overall more complicated study design involving four different emotions instead of only one or two. I therefore wanted to investigate possible order or context effects and to determine whether the neural response to facial expressions of a specific emotion remains constant regardless of whether the expressions were presented first, or preceded by a different emotion and being presented as the second, third or fourth experiment a subject performed.

In this chapter I will describe four separate analyses and studies: firstly, a re-analysis of the data presented in chapter 3; secondly a further re-analysis of those data together with data from Phillips et al (1998b); thirdly I will describe a study looking at the effects of context on the neural responses to facial expressions of disgust; and lastly I will present a study investigating the effects of order on the neural responses to facial expressions of disgust.

4.2 Re-analysis of data presented in chapter 3

4.2.1 Methods

The data presented in chapter 3 were re-analysed by dividing up the subjects according to whether they had been presented with the facial expression of a specific emotion (anger, disgust, fear or sadness) as the first experiment or as experiment 2, 3 or 4. This is illustrated in table 4.2.1.

Table 4.2.1: Grouping of subjects for re-analysis of data

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Subject 1	Fear-vis	Sadness-aud	Fear-aud	Sadness-vis
Subject 2	Sadness-aud	Sadness-vis	Fear-vis	Fear-aud
Subject 3	Sadness-vis	Fear-vis	Fear-aud	Sadness-aud
Subject 4	Anger-vis	Fear-aud	Fear-vis	Anger-aud
Subject 5	Fear-aud	Anger-aud	Anger-vis	Fear-vis
Subject 6	Fear-vis	Anger-aud	Anger-vis	Fear-aud
Subject 7	Disgust-vis	Fear-vis	Fear-aud	Disgust-aud
Subject 8	Fear-aud	Fear-vis	Disgust-aud	Disgust-vis
Subject 9	Disgust-vis	Fear-aud	Disgust-aud	Fear-vis

In this table the grouping of subjects according to whether they were exposed to facial expressions of a specific emotion in experiment 1 or in experiment 2-4 is shown using expressions of fear as an example. Only 9 subjects are shown as the other 9 subjects from chapter 3 were not presented with expressions of fear. Subjects who were presented with facial expressions of fear in experiment 1 are shown in red, subjects who were presented with facial expressions of fear in experiment 2-4 are shown in blue. One subject (No. 8) was presented with facial expressions of fear after being presented with vocal expressions of fear, this subject was excluded from the re-analysis. The same principle was used to group presentations of facial expressions of disgust.

Group sizes for this re-analysis were as follows:

Facial expressions of anger presented in experiment 1: N = 2

Facial expressions of anger presented in experiment 2-4: N = 6

Facial expressions of disgust presented in experiment 1: N = 3

Facial expressions of disgust presented in experiment 2-4: N = 5

Facial expressions of fear presented in experiment 1: N = 2

Facial expressions of fear presented in experiment 2-4: N = 6

Facial expressions of sadness presented in experiment 1: N = 2

Facial expressions of sadness presented in experiment 2-4: N = 5

4.2.2 Results – Generic brain activation mapping

Table 4.2.2a: Major regions of generic brain activation in response to facial expressions of anger

Cerebral Region (BA)	Side	Tal(x)	Tal(y)	Tal(z)	Size
Anger experiment 1					
Cerebellum	L/R	-15	-73	-29	51
		21	-73	-29	40
Primary Visual Cortex (BA 18)	L/R	0	-73	-2	32
		-7	-80	15	19
Inferior Temporal Gyrus (BA 20)	R	28	-20	-29	11
Occipitotemporal Gyrus (BA 36)	R	25	-17	-24	11
Auditory Cortex (BA 42)	L	-57	-30	9	10
Insula	R	43	-30	4	9
Superior Temporal Gyrus (BA 22)	L	-57	-17	4	7
Anger experiment 2-4					
Cerebellum	L/R	4	-69	-13	38
		-17	-63	-7	28
Ventrolateral Prefrontal Cortex (BA 47)	R	40	20	-13	28
Middle Temporal Gyrus (BA 21)	L	-47	-10	-7	16
Primary Visual Cortex (BA 18)	L/R	4	-82	-2	15
		-15	-73	26	11
Medial Frontal Lobe (BA32)	R	7	10	42	13
Inferior Temporal Gyrus (BA20)	L	-40	-13	-29	12
Thalamus	R	4	-33	4	11
Premotor Cortex & SMA (BA 6)	L	-25	-7	48	9
Post Cingulate Cortex (BA 30)	R	0	-50	20	7
Inferior Frontal Gyrus (BA 45)	L	-40	17	20	5

Anger experiment 1 shows the Talairach coordinates of the neural responses to facial expressions of anger presented in experiment 1. Anger experiment 2-4 shows the Talairach coordinates of the neural responses to facial expressions of anger presented in experiment 2-4.

Table 4.2.2b: Major regions of generic brain activation in response to facial expressions of disgust

Cerebral Region (BA)	Side	Tal(x)	Tal(y)	Tal(z)	Size
Disgust experiment 1					
Primary Visual Cortex (BA 19)	L	-40	-63	31	9
Angular Gyrus	L	-40	-63	26	8
Disgust experiment 2-4					
Post Cingulate Gyrus (BA 31)	R	0	-52	26	22
Hippocampus	R	21	-10	-13	18
Dorsolateral Prefrontal Cortex (BA 9)	L	-32	26	26	11
Premotor Cortex & SMA (BA 6)	L	-40	4	37	10
Primary Visual Cortex (BA 19)	L	-32	-60	37	10
Inferior Temporal Lobe (BA 34)	L	-17	7	-13	5

Disgust experiment 1 shows the Talairach coordinates of the neural responses to facial expressions of disgust presented in experiment 1. Disgust experiment 2-4 shows the Talairach coordinates of the neural responses to facial expressions of disgust presented in experiment 2-4.

Table 4.2.2c: Major regions of generic brain activation in response to facial expressions of fear

Cerebral Region (BA)	Side	Tal(x)	Tal(y)	Tal(z)	Size
Fear experiment 1					
Primary Visual Cortex (BA 18/19)	L/R	-28	-69	31	17
		28	-80	-7	7
Inf-Post Temporal Lobe	L/R	-40	-52	-18	11
		47	-50	-18	8
Post Cingulate Cortex (BA 30)	L	-25	-43	-2	7
Occipitotemporal Gyrus (BA 36)	R	25	-23	-24	7
Inferior Frontal Gyrus (BA 45)	L	-47	17	20	5
Fear experiment 2-4					
Retrosplenial Cortex (BA 27/35)	L/R	11	-33	-2	15
		-17	-17	-24	7
Cerebellum	L/R	11	-43	-7	10
		-17	-30	-18	7
Insula	L	-28	30	4	9

Fear experiment 1 shows the Talairach coordinates of the neural responses to facial expressions of fear presented in experiment 1. Fear experiment 2-4 shows the Talairach coordinates of the neural responses to facial expressions of fear presented in experiment 2-4.

Table 4.2.2d: Major regions of generic brain activation in response to facial expressions of sadness

Cerebral Region (BA)	Side	Tal(x)	Tal(y)	Tal(z)	Size
Sadness experiment 1					
Anterior Cingulate Gyrus (BA 24)	R	0	7	37	10
Angular Gyrus (BA 39)	L	-43	-56	31	8
Middle Temporal Gyrus (BA 21)	L	-53	-23	-7	8
Insula	L	-25	13	4	7
Posterior Cingulate Gyrus (BA 31)	R	4	-50	26	7
Inferior Temporal Lobe (BA 34)	L	-21	13	-13	6
Medial Frontal Lobe (BA 32)	R	7	33	26	6
Sadness experiment 2					
Inferior-Posterior Temporal Lobe (BA37)	R	43	-52	-18	28
Cerebellum	L/R	36	-52	-24	27
		-25	-50	-24	8
Auditory Cortex (BA 42)	L	-40	-30	20	18
Middle Temporal Gyrus (BA 21)	R	50	-20	-7	16
Superior Temporal Gyrus (BA 22)	L	-40	-23	15	12
Primary Visual Cortex (BA 18)	R	7	-67	26	9
Supramarginal Gyrus (BA 40)	L	-43	-30	26	8
Dorsolateral Prefrontal Cortex (BA 9)	L	-28	4	42	8

Sadness experiment 1 shows the Talairach coordinates of the neural responses to facial expressions of sadness presented in experiment 1. Sadness experiment 2-4 shows the Talairach coordinates of the neural responses to facial expressions of sadness presented in experiment 2-4.

4.2.3 Discussion

In this exploratory analysis the group sizes for emotions presented first were very small (2-3 subjects), for emotions presented 2^{nd} – 4^{th} the group size was 5-6 subjects. A group size of only 2-3 subjects is too small to yield meaningful results and there is not sufficient statistical power.

4.3 Re-analysis including data from Phillips et al.

Phillips et al. had employed the same stimuli, experimental design and acquisition parameters to investigate the neural correlates of facial expressions of fear and disgust (Phillips et al., 1998b). I therefore combined these data with mine in order to increase the group size for the very first experiment a subject was exposed to. Unfortunately this had to be limited to fear and disgust as Phillips et al. did not investigate the neural correlates of anger and sadness.

4.3.1 Methods

The combined data from chapter 3 and from Phillips et al. (1998b) was divided up the same way as in section 4.2.1.

Group sizes for this re-analysis were as follows:
Facial expressions of disgust presented in experiment 1: N = 5
Facial expressions of disgust presented in experiment 2-4: N = 9
Facial expressions of fear presented in experiment 1: N = 3
Facial expressions of fear presented in experiment 2-4: N = 10

4.3.2 Results – Generic brain activation mapping

There was hardly any activation in response to facial expressions of disgust presented first (figure 4.3.2a and table 4.3.2a). The only regions activated in response to both disgust presented first and presented in experiments 2-4 were the hippocampus and the inferior posterior temporal lobe (BA 37).

The main areas of activation in response to facial expressions of fear presented first were visual areas, and the posterior cingulate gyrus (BA 31) was the only area thought to be involved in emotion processing (Maddock, 1999) that was activated (figure 4.3.2b and table 4.3.2b). Apart from visual areas the only region activated in reponse to both fear presented in experiment 1 and in experiments 2-4 was the inferior posterior temporal cortex (BA 37).

However, when looking at the results for disgust and fear presented as the second, third or fourth experiment, an unusual finding became evident. Instead of the expected insula activation in response to facial expressions of disgust and amygdala activation in response to facial expressions of fear the pattern was reversed (figure 4.3.2a & b, table 4.3.2a & b), with amygdala activation in response to facial expressions of disgust and insula and putamen activation in response to facial expressions of fear.

Table 4.3.2a: Major regions of generic brain activation in response to facial expressions of disgust

Cerebral Region (BA)	Side	Tal(x)	Tal(y)	Tal(z)	Size
Digust Experiment 1					
Inf-Posterior Temporal Lobe (BA 37)	R	53	-50	-2	5
Hippocampus	R	32	-20	-7	3
Angular Gyrus (BA 39)	L	-47	-56	26	3
Disgust Experiment 2-4					
Temporal Lobe (Uncus) (BA 34)	R	17	-4	-13	22
Hippocampus	R	28	-13	-18	13
Putamen	R	25	-4	4	9
Cerebellum	L	-21	-67	-24	8
Inferior Frontal Gyrus (BA 45)	L	-40	17	20	8
Post Cingulate Gyrus (BA 31)	R	0	-63	20	7
Amygdala	L	-25	-7	-13	6
Inf-Post Temporal Lobe (BA 37)	L	-40	-56	9	6
Dorsolateral Prefrontal Cortex (BA 9)	L	-36	20	26	6
Superior Temporal Gyrus (BA 22)	R	57	-33	4	6

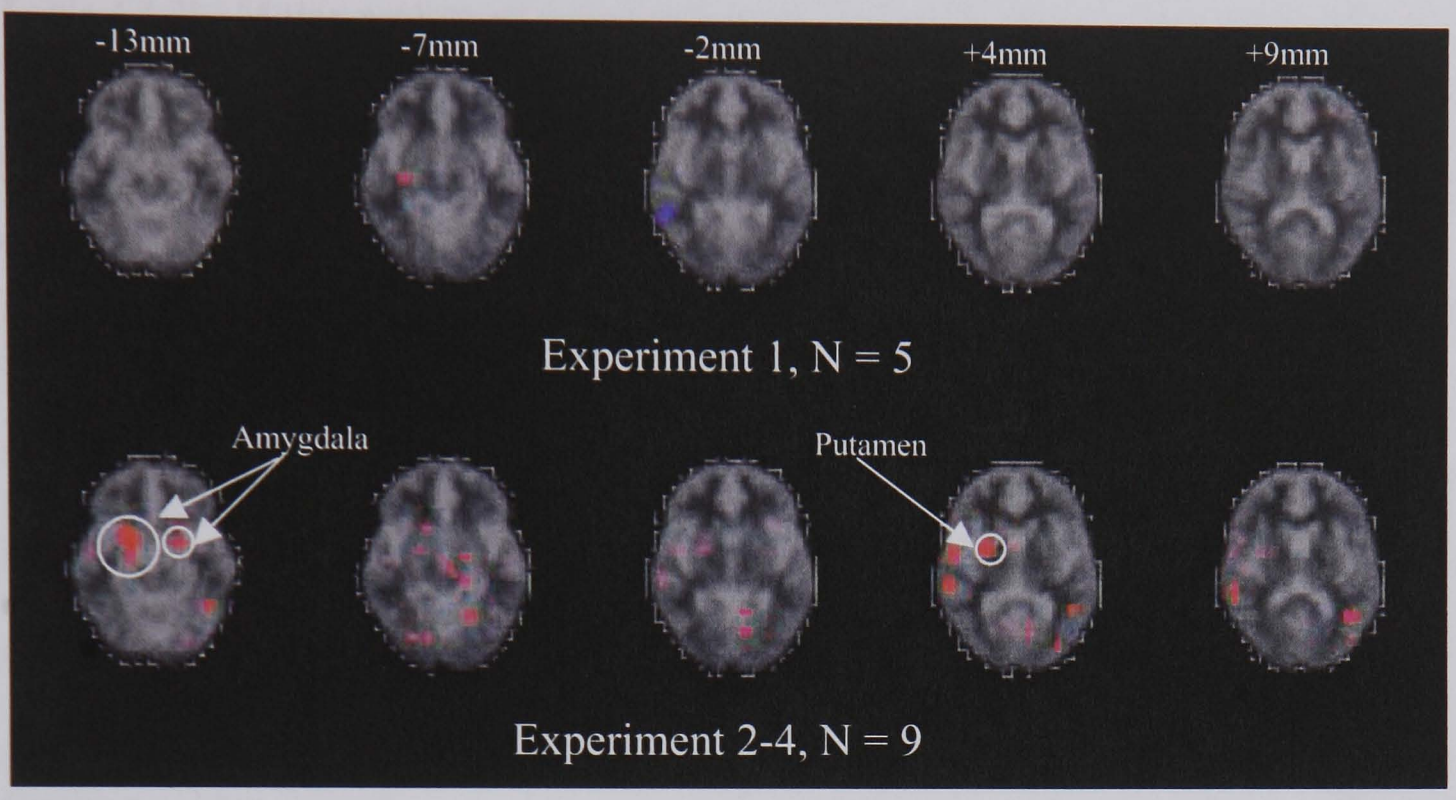


Figure 4.3.2a: Major regions of generic brain activation in response to facial expressions of disgust

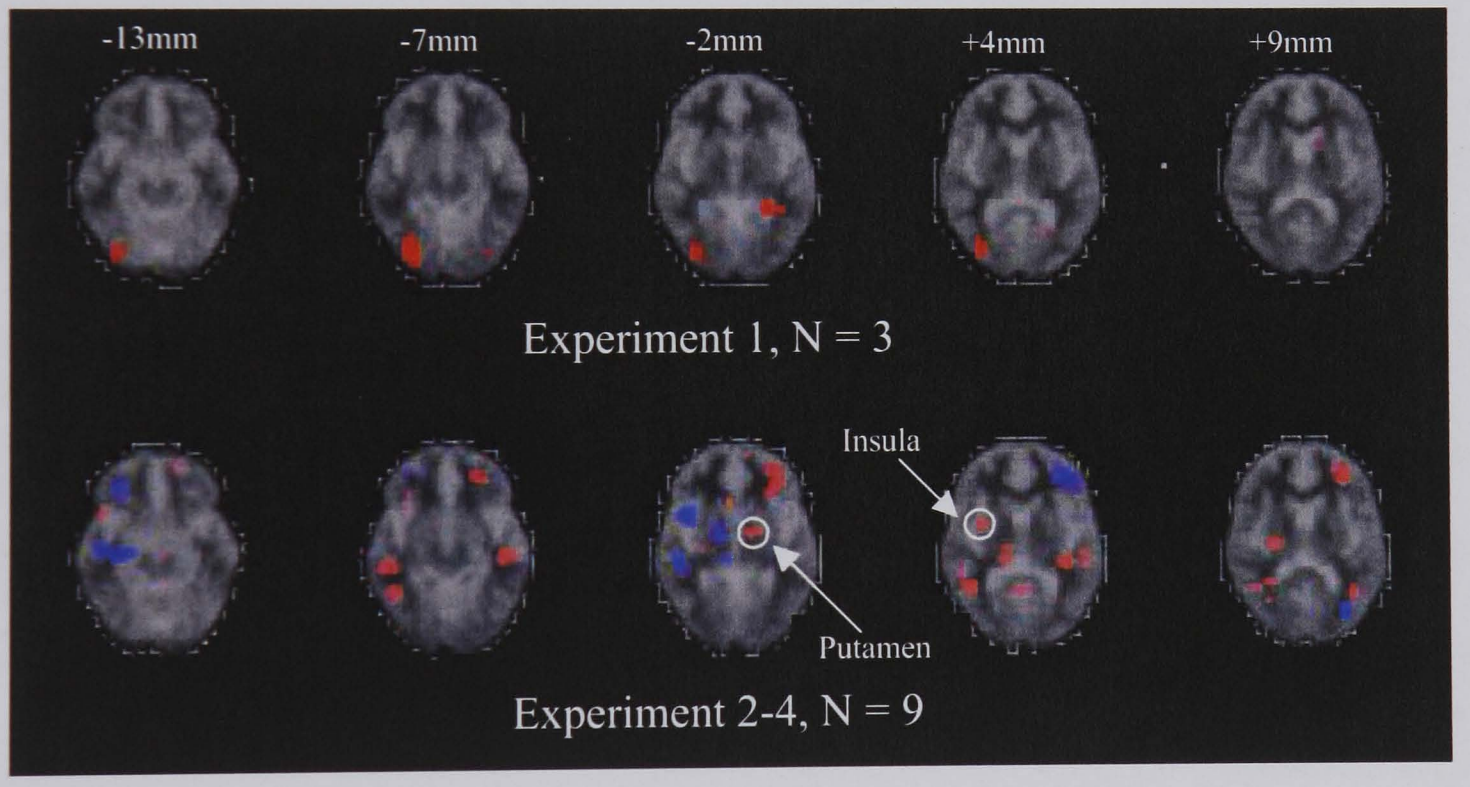


Figure 4.3.2b: Major regions of generic brain activation in response to facial expressions of fear

In both figures the right side of the brain is shown on the left side of each panel, and vice versa. The numbers above the images indicate the distance in mm from the transcallosal line. Voxels activated at $p<0.004$ by facial expressions of disgust are shown in red, and voxel activated at $p<0.004$ during the neutral condition are shown in blue.

Table 4.3.2b: Major regions of generic brain activation in response to facial expressions of fear

Cerebral Region (BA)	Side	Tal(x)	Tal(y)	Tal(z)	Size
Fear Experiment 1					
Post Cingulate Gyrus (BA 31)	L	-17	-56	26	20
Primary Visual Cortex (BA 19)	L/R	-32	-69	31	18
		36	-76	-13	6
Primary Visual Cortex (BA 18)	R	28	-80	-7	17
Parietal Association Cortex (BA 7)	L	-21	-52	42	8
Inferior Temporal Gyrus (BA 20)	R	25	-23	-29	6
Cerebellum	L	-7	-73	-18	6
Inf-Post Temporal Lobe (BA 37)	R	36	-69	4	6
Fear Experiment 2-4					
Parahippocampal Gyrus	L/R	40	-23	-13	26
		-36	-33	4	7
Ventrolateral Prefrontal Cortex (BA 47)	L/R	-32	33	-2	24
		32	26	-13	9
Insula	R	40	4	-2	13
Inferior Temporal Gyrus (BA 20)	L	-17	-26	-18	18
Primary Visual Cortex (BA 18)	L	-21	-69	26	16
Middle Temporal Gyrus (BA 21)	R	47	-33	-2	10
Putamen	L/R	17	-7	-2	8
		-15	-10	-2	5
Inf-Post Temporal Lobe (BA 37)	R	43	-52	-7	8
Primary Visual Cortex (BA 19)	L	-28	-73	9	8
Medial Frontal Lobe (BA 32)	L	-21	33	-7	6
Retrosplenial Cortex (BA 35)	R	43	-17	-18	6
Superior Temporal Gyrus (BA 22)	R	32	-39	15	6
Supramarginal Gyrus (BA 40)	L	-36	-39	26	6
Thalamus	R	25	-23	9	5

4.3.3 Discussion

Despite the inclusion of the data of Phillips et al., the group size of subjects who were exposed to a specific experiment first was still relatively small (N = 3-5). This meant that, although the data for emotions presented 2^{nd} – 4^{th} were reliable now with group sizes of up to 9 subjects, it was difficult to draw conclusions as it could not reliably be established whether those results differed from the results for the same emotion presented first. However, despite a group size of 5 subjects there was no insula activation in response to facial expression of disgust in experiment 1. I expected to see insula activation even for this relatively small group size as Phillips et al. observed insula activation in response to facial expressions of disgust with relatively small group sizes of 6 (Phillips et al., 1998b) and 7 (Phillips et al., 1997) subjects.

The hippocampus was activated by all experiments presenting facial expressions of disgust. Hippocampal activation has been demonstrated in a previous study during primary visual processing of the face (Kapur et al., 1995). It is unclear why this activation was not observed in response to facial expressions of fear in this study, especially as this has been reported before (Fischer et al., 2003). The only region activated in response to all experiments was the inferior posterior temporal lobe (BA 37), which is part of the occipitotemporal visual pathway. Previous neuroimaging studies have reported activation in this area in response to a variety of visual stimuli, including mouth movements (Campbell et al., 2001), and face perception (Adolphs, 2002; Haxby et al., 2000).

The pattern of results for facial expressions of fear and disgust presented 2^{nd} – 4^{th} is highly unusual. Instead of insula activation in response to facial expressions of disgust and amygdala activation in response to facial expressions of fear this result is reversed. These groups of subjects had been exposed to a variety of different emotions in both the visual and auditory modality before viewing facial expressions of fear or disgust. This indicates that the context in which facial expressions of emotion are presented might influence the neural structures involved in the perception of those emotions.

4.4 Investigation of context effects

As the group sizes for subjects being presented first with a specific emotion were still quite small I performed a new study investigating the effect that order of experiments plays in the results, to determine for example, if the result is the same regardless of whether subjects are exposed to facial expressions of disgust first or whether they are exposed to facial expressions of disgust after they have seen facial expressions of other emotions (anger, fear and sadness). This study was performed in conjunction with the study described in section 4.5, which establishes activation in response to facial expressions of disgust presented first and acts as a control for the study presented in this section. The results of the relevant comparisons are presented in section 4.5.5.4. Based on the results of chapter 3 it is hypothesized that the order in which subjects are exposed to different emotions will influence the neural responses to the target emotion, in this case disgust.

4.4.1 Subjects

6 right-handed, male volunteers (mean age 31 years; mean NART IQ estimate 119.8, based on 5 subjects as one subject was not a native speaker; mean time spent in full time education since the age of 5, 19.3 years) participated in four experiments in the same testing session. Exclusion criteria were history of brain injury and past and current psychiatric illness. Informed written consent was obtained from all subjects.

4.4.2 Stimuli

The stimuli depicting black-and-white grey scale images of prototypical facial expressions of disgust or neutral were taken from a standard series (Ekman & Friesen, 1976) of pictures of facial expressions of emotion containing male and female faces. These have been described in more detail in section 3.2.3 and have been used in previous studies (Phillips et al., 1997, 1998b, 2000).

4.4.3 Experimental Design

Each subject performed 4 different 5-min experiments. As in chapter 3, each experiment comprised five cycles of periodic alternation between 30s epochs of emotion (anger, fear and sadness in experiments 1-3 and disgust in experiment 4) and neutral facial expressions. In each 30s epoch eight different facial identities displaying either a disgusted or a neutral facial expression were each presented for 3s, with an interstimulus interval of 0.75s. During each experiment the subjects performed a sex-decision task, as in chapter 3 and were unaware of the emotional focus of this study. The order of anger, fear and sadness was counterbalanced between subjects, but every subject was exposed to facial expressions of disgust in experiment 4 (table 4.4.3). This was to determine whether being exposed to other emotions before seeing facial expressions of disgust has an influence on the neural correlates involved in the perception of facial expressions of disgust. After scanning, subjects were asked to identify the facial expressions by choosing one out of 7 (anger, disgust, fear, happiness, neutral, sadness, surprise) possible emotions for each stimulus.

Table 4.4.3: Experimental design

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Subject 1	Anger	Fear	Sadness	Disgust
Subject 2	Anger	Sadness	Fear	Disgust
Subject 3	Fear	Anger	Sadness	Disgust
Subject 4	Fear	Sadness	Anger	Disgust
Subject 5	Sadness	Anger	Fear	Disgust
Subject 6	Sadness	Fear	Anger	Disgust

4.4.4 Data analysis

Data were analysed using generic brain activation mapping as described in section 2.4.1.

4.4.5 Results

4.4.5.1 Facial expression recognition

The subjects performed at a level of 92.5 - 100% correct in the sex-decision task. There was no reduction in accuracy scores over the course of the scanning session, demonstrating that subjects were attentive throughout the study. In the post-scan questionnaire, 75% of the disgust faces were correctly identified as disgust, 72.5 % of angry faces, 60% of fearful faces and 75% of sad faces were correctly identified, and 98% of the neutral faces were correctly identified as neutral. On this task subjects ranged from 57.5% - 97.5% correct.

4.4.5.2 Generic brain activation maps

Only the neural responses to facial expressions of disgust presented as the last of four experiments were analysed. As I hypothesized that the order of experiments would influence the result the focus was on this last experiment, and for anger, fear and sadness the group size of a specific emotion presented as the first experiment was too small (N = 2) to yield meaningful results (table 4.4.3).

When subjects were exposed to facial expressions of disgust after they had already seen facial expressions of anger, fear and sadness the only brain regions that showed an increase in activation were the cerebellum, the medial frontal lobe (BA 32) and the premotor cortex (BA 6) (table 4.4.5.2).

Table 4.4.5.2: Main generically activated brain regions in response to facial expressions of disgust presented last.

Cerebral Region (Brodmann area)	Side	Tal(x)	Tal(y)	Tal(z)	Size	Emotion
Fusiform Gyrus (BA 37)	R	25	-56	-13	25	Disgust
Medial Frontal Lobe (BA 32)	R	7	30	26	5	Disgust
Premotor Cx & SMA (BA 6)	R	7	-13	48	4	Disgust

4.4.6 Discussion

There was no insula activation in response to facial expressions of disgust when subjects had viewed facial expressions of anger, fear and sadness before viewing facial expressions of disgust. This could be due to an influence of the context in which emotional expressions are viewed on the neural response. Another reason for the apparent lack of insula activation could be habituation of brain regions involved in perception of facial expressions of emotion, which could also affect neural activation in regions involved in the perception of a specifc emotion.

4.5 Investigation of order effects

In the preceding study investigating potential context effects facial expressions of disgust were always presented last. Consequently, the results could have been influenced not only by the context but also by the order. I therefore performed another study, this time focussing on the effects of order. Based on previous results from the amygdala (Breiter et al., 1996; Phillips et al., 2001) and on more general habituation literature I therefore predict a change in neural response to repeated presentations of facial expressions of disgust, specifically a reduction in the activation of the insula.

4.5.1 Subjects

6 right-handed, male volunteers (see table 4.5.1 for subject details) participated in four experiments in the same testing session. The groups in sections 4.4 and 4.5 are homogeneous as regards age and NART (Mann-Whitney U test, $p > 0.1$). However, they are significantly different (table 4.5.1) with regard to time spent in full time education since the age of 5 (Mann-Whitney U test, $p < 0.05$). Exclusion criteria were history of brain injury and past and current psychiatric illness. Informed written consent was obtained from all subjects.

Table 4.5.1: Subject details for the groups described in section 4.4 and 4.5
All information is given as mean (± STD).

	Subjects from section 4.4	Subjects from section 4.5
Age	31 (± 8.2)	28.2 (± 7.1)
Time in Full-time education	19.3 (± 2.2)	16.5 (± 2) *
NART IQ estimate	119.8 (± 6.3)	117.8 (± 6.9)

* significant difference, $p < 0.05$

4.5.2 Stimuli

The stimuli of disgusted and neutral facial expressions used in this study have been described in detail in section 3.2.3. and have been used in previous studies (Phillips et al., 1997, 1998b, 1999).

4.5.3 Experimental Design

Each subject performed the same 5-min experiment 4 consecutive times. These successive presentations are referred to as Experiments 1 to 4. The design of each 5-minute experiment is identical to the design in chapter 3, each experiment comprised five cycles of periodic alternation between 30s epochs of disgust and neutral facial expressions. The stimulus presentation was the same as for section 4.4, as was the sex-decision task. After scanning, subjects were asked to identify the facial expressions by choosing one out of 7 (anger, disgust, fear, happiness, neutral, sadness, surprise) possible emotions for each stimulus.

4.5.4 Data analysis

Data were analysed using generic brain activation mapping as described in section 2.4.1. To estimate the differences in mean fundamental power quotient (FPQ) between experiments 1-4 the same ANCOVA as described in section 3.2.5 was performed.

Experiment 1 was performed to establish the baseline pattern of activation in response to facial expressions of disgust, and subsequent experiments were performed to monitor changes over time in response to repeated presentation of the same stimuli. For this reason, I limited the number of statistical comparisons to three: I compared activation during Experiments 2, 3 and 4 each with the activation observed at baseline, Experiment 1.

Table 4.5.3: Experimental design

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Subject 1	Disgust	Disgust	Disgust	Disgust
Subject 2	Disgust	Disgust	Disgust	Disgust
Subject 3	Disgust	Disgust	Disgust	Disgust
Subject 4	Disgust	Disgust	Disgust	Disgust
Subject 5	Disgust	Disgust	Disgust	Disgust
Subject 6	Disgust	Disgust	Disgust	Disgust

This table shows all six subjects viewing facial expressions of disgust in all four experiments.

4.5.5 Results

4.5.5.1 Facial expression recognition

The subjects performed at a level of 92.5-100% correct in the sex-decision task. Due to a technical fault when the computer did not record the responses there were no behavioural data for one subject. There was no reduction in accuracy scores over the course of the scanning session, demonstrating that subjects were attentive throughout the study. In the post-scan questionnaire, 83.5% of the disgust faces were correctly identified as disgust, and 98% of the neutral faces were correctly identified as neutral, results which are consistent with previously reported recognition rates for these emotional expressions (Ekman & Friesen, 1976). Subjects ranged from 62.5 – 97.5% correct on this task.

4.5.5.2 Generic brain activation maps

For group 1, in the first experiment (figure. 4.5.5.2, Disgust 1; table 4.5.5.2a) major regions of activation in response to facial expressions of disgust were observed in the insula, primary visual (peristriate) cortex (BA 18), anterior cingulate gyrus (BA 24), and orbitofrontal cortex (BA 11). There was no activation in response to neutral faces. This pattern of activation remained similar in experiment 2 (figure 4.5.5.2, Disgust 2; table 4.5.5.2a), in which major regions of activation in response to disgust were observed in the anterior cingulate gyrus (BA 24) and insula, and also within the medial frontal (BA 32), and precentral (BA 4) gyri. Activation in response to neutral faces occurred in visual areas, including primary visual cortex (BA19) and the angular gyrus (BA39). In experiment 3 (figure 4.5.5.2, Disgust 3; table 4.5.5.2b), activation occurred predominantly in response to neutral faces rather than expressions of disgust: in the insula, anterior cingulate gyrus (BA 24), thalamus, cerebellum, precentral gyrus (BA 4) and posterior cingulate gyrus (BA 31). In the final experiment (figure 4.5.5.2, Disgust 4; table 4.5.5.2b) activation in response to disgust occurred in the hippocampus. Activation in response to neutral faces occurred in the cerebellum, orbitofrontal cortex, and visual processing regions (BA 18).

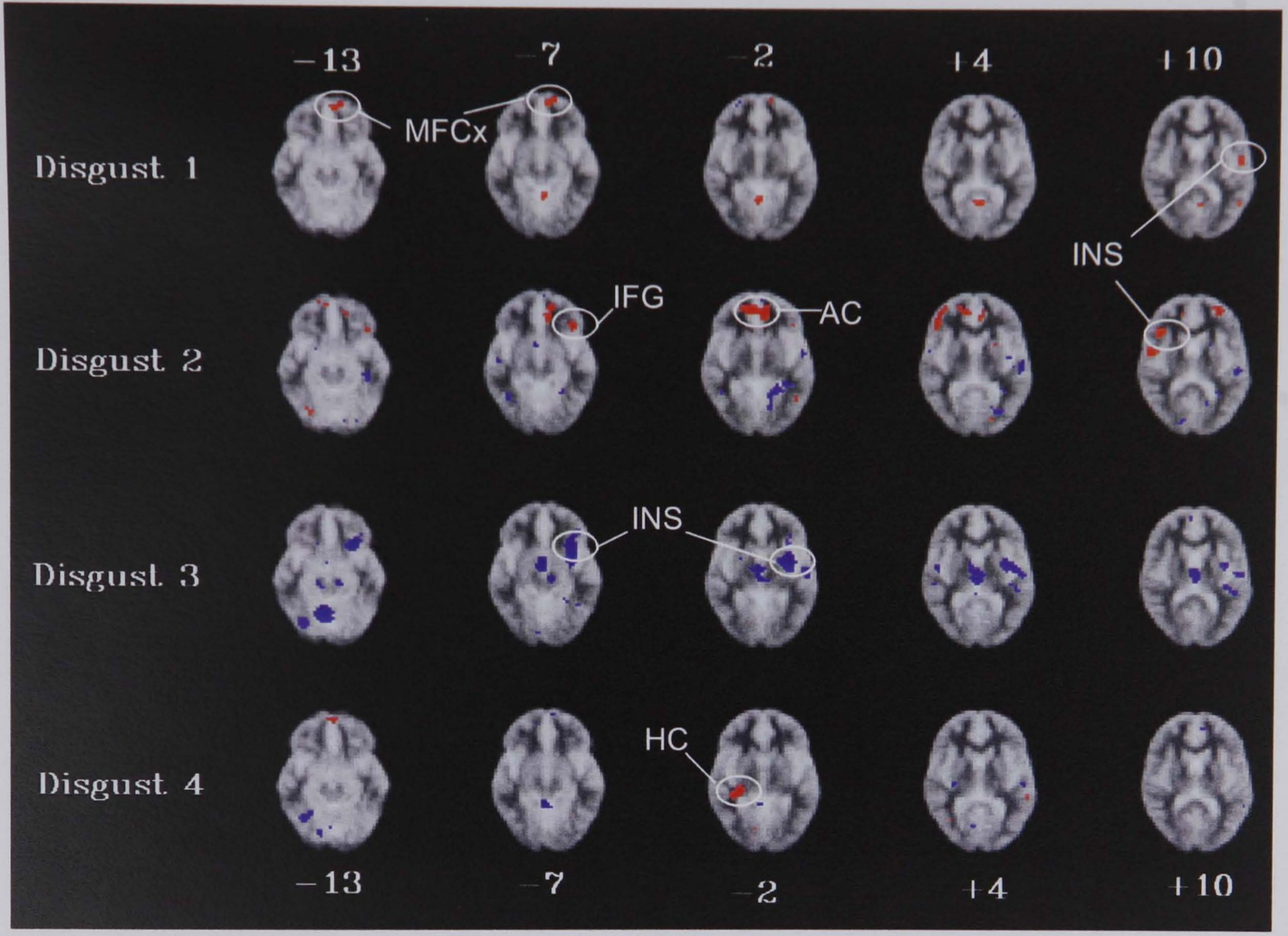


Figure 4.5.5.2: Foci of generic brain activation in six right-handed normal male subjects during repeated perception of facial expressions of disgust.

The numbers above and below the transverse sections indicate the distance in mm from the transcallosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by facial expressions of disgust are shown in red, and voxel activated at $p<0.004$ during the neutral condition are shown in blue.

MFCx = Medial frontal cortex, AC = anterior cingulate, IFG = inferior frontal gyrus, INS = insula, HC = hippocampus

Table 4.5.5.2a: Main generically activated brain regions in response to repeated presentation of facial expressions of disgust.
Showing experiment 1 and experiment 2.

Cerebral Region (Brodmann area)	Side	Tal(x)	Tal(y)	Tal(z)	Size	Emotion
Disgust 1						
Medial Frontal Lobe (32)	R	7	13	42	7	Disgust (D)
Frontal Pole (10)	L	-15	46	-7	7	D
Primary Visual Cortex (18)	R/L	0	-56	4	4	D
Anterior Cingulate Gyrus (24)	L	-4	10	26	4	D **
Insula	L	-47	-10	9	4	D
Disgust 2						
Anterior Cingulate Gyrus (24)	L	-7	33	-2	30	D
Medial Frontal Lobe (32)	R/L	0	26	26	22	D
Angular Gyrus (39)	L	-40	-63	26	15	D
Insula	R	43	20	4	13	D
		40	13	9	8	D
Premotor Cortex & SMA (6)	L	-43	-10	26	13	D
Inferior frontal gyrus (44/47)	R	47	10	20	8	D
	L	-36	20	-7	7	D
Angular Gyrus/Inferior Parietal Lobule (39/40)	R/L	40	-56	31	44	Neutral (N)
		-40	-52	42	26	
Anterior Cingulate Gyrus (24)	R	7	-23	37	11	N
Precuneus (7)	R	28	-56	37	30	N
	L	-21	-60	48	17	N
Primary Visual Cortex (19)	L	-21	-50	-2	28	N
	R	28	-69	26	24	N
Medial Frontal Lobe (32)	L	-21	39	15	12	N
Post Cingulate Gyrus (23/31)	R	15	-23	42	13	N
	L	-15	-60	15	7	N

The last column (Emotion) indicates whether the region was activated in response to disgusted (D) faces or in response to neutral (N) faces.
* - areas in which there was significantly greater activation than in experiment 1, p = 0.01;
** - area in which there was significantly greater activation in experiment 1 than in experiment 4, p = 0.01.

Table 4.5.5.2b: Main generically activated brain regions in response to repeated presentation of facial expressions of disgust.
Continued from table 4.5.5.2a, showing experiment 3 and experiment 4.

Cerebral Region (Brodmann area)	Side	Tal(x)	Tal(y)	Tal(z)	Size	Emotion
Disgust 3						
Precentral Gyrus (4)	L	-47	-4	37	10	D
Inferior Frontal Gyrus (44)	L	-47	0	31	8	D
Precuneus (7)	R	4	-52	37	45	N
	L	-4	-43	48	5	N
Insula	L	-28	13	-7	44	N *
		-32	-10	-2	31	N *
Cerebellum	R	7	-60	-13	31	N
Thalamus	R/L	0	-23	4	28	N *
Anterior Cingulate Gyrus (24)	R	7	10	37	26	N
	L	-7	0	42	7	N
Post Cingulate Gyrus (31)	R/L	0	-50	26	25	N
Temporal Lobe (Uncus) (34)	L	-25	13	-13	21	N
Medial Frontal Lobe (32)	R/L	0	26	26	19	N
Angular Gyrus (39)	L	-36	-60	26	19	N
Superior Temporal Gyrus (22)	L	-36	-33	9	15	N *
Parahippocampal Gyrus (28)	R	17	-20	-18	14	N
Inferior Frontal Gyrus (47)	L	-21	13	-18	11	N
Premotor Cortex & SMA (6)	L	-47	-4	26	10	N
Disgust 4						
Hippocampus	R	32	-33	-2	7	D *
Cerebellum	R	36	-60	-13	7	N *
Primary Visual Cortex (18)	R	11	-73	26	7	N *

The last column (Emotion) indicates whether the region was activated in response to disgusted (D) faces or in response to neutral (N) faces.
* - areas in which there was significantly greater activation than in experiment 1, $p = 0.01$;
** - area in which there was significantly greater activation in experiment 1 than in experiment 4, $p = 0.01$.

4.5.5.3 Comparison of activation observed between baseline and subsequent experiments

Experiments 1 and 2.

Significantly more activation ($p = 0.01$) occurred in experiment 2 than in experiment 1 in the inferior frontal gyrus (BA 45) but not the insula in response to facial expressions of disgust, and in the posterior cingulate gyrus in response to neutral faces (search volume = 746 voxels, expected number of false positive voxels = 7, observed number of voxels with significantly greater activation in experiment 2 than experiment 1 = 96). No regions were activated to a significantly greater extent to either disgust or neutral facial expressions in experiment 1 than in experiment 2 (table 4.5.5.2a).

Experiments 1 and 3.

There were no regions activated to a significantly greater extent in experiment 3 than in experiment 1 in response to facial expressions of disgust. However, significantly greater activation ($p = 0.01$) was demonstrated in experiment 3 than in experiment 1 in response to neutral faces in the insula, thalamus and putamen (search volume = 794 voxels, expected number of false positive voxels = 7, observed number of voxels with significantly greater activation in experiment 3 than experiment 1 = 181). No regions were activated to a significantly greater extent to either disgust or neutral facial expressions in experiment 1 than in experiment 3 (table 4.5.5.2a and table 4.5.5.2b).

Experiment 1 and 4.

In experiment 4, in comparison with experiment 1, significantly greater activation ($p = 0.01$) occurred in the hippocampus in response to disgust faces, and in primary visual areas and the cerebellum to neutral faces; the anterior cingulate gyrus (BA 24) was activated to a significantly greater extent ($p = 0.01$) in experiment 1 than in experiment 4 (search volume = 218 voxels, expected number of false positive voxels = 2, observed number of voxels with significantly greater activation in experiment 1 than experiment 4 = 6, observed number of voxels with significantly greater activation in experiment 4 than experiment 1 = 21).

The Talairach coordinates of the most significant differences for all three comparisons are marked with asterisks in table 4.5.5.2a and table 4.5.5.2b.

4.5.5.4 Between-group comparison

In order to determine whether there were differences in activation between the response to facial expressions of disgust preceded by facial expression of anger, fear and sadness (section 4.4) and the response to facial expressions of disgust preceded by 3 experiments showing facial expressions of disgust (section 4.5), I compared the results for experiment 4 (see tables 4.4.3 and 4.5.3) in both groups. The comparison was made using the same technique as described in section 3.2.5.

At a voxel-wise p = 0.001 there was no statistically significant difference between the neural response to facial expressions of disgust presented after facial expressions of anger, fear and sadness and to facial expressions of disgust presented last in a series of 4 experiments all showing facial expressions of disgust. This suggests that preceding an experiment investigating perception of facial expressions of a target emotion with presentations of facial expressions of different emotions has the same effect as preceding it with presentations of facial expressions of the same emotion. It is unclear therefore what role context plays as compared to the role of repeated stimulus presentation.

In order to establish if there are statistically significant differences in activation between facial expressions of disgust presented first (section 4.5) and facial expressions of disgust presented subsequent to presentation of facial expressions of anger, fear and sadness (section 4.4), the results for experiment 1 from section 4.5 were compared with the results of experiment 4 from section 4.4. The results were compared as described in section 3.2.5.

The results of this comparison are shown in table 4.5.5.4. Activation in response to facial expressions of disgust presented first is greater than activation in response to facial expressions of disgust preceded by facial expressions of anger, fear and sadness in all areas which were originally observed in the generic brain activation map (figure 4.5.5.2 and tables 4.5.5.2a&b), except for activation in the insula, where there is no statistically significant difference (p = 0.05, 5 observed cluster, 0.65 expected false-positive clusters). The only region that is activated to a greater extent when facial expressions of disgust are presented following facial expressions of anger, fear and

sadness as compared to facial expressions of disgust presented first is the occipitotemporal junction (BA 37) (p = 0.001, 1 observed cluster, 0 expected false-positive clusters).

Table 4.5.5.4: Comparison between facial expressions of disgust presented first and preceeded by facial expressions of anger, fear and sadness.

Cerebral Region (Brodmann area)	Side	Tal(x)	Tal(y)	Tal(z)	Size	Cluster-wise p value
Disgust 1 > 4						
Frontal Pole (BA 10)	L	-9	58	-10	16	0.05
Anterior Cingulate Gyrus (BA 24)	L	-1	17	30	13	0.05
Medial Frontal Lobe (BA 32)	L	-16	36	37	10	0.05
	R	8	22	42	8	
Primary Visual Cortex (BA 18)	L	-1	-58	5	7	0.05
Disgust 4 > 1						
Fusiform Gyrus (BA 37)	R	38	-51	-13	11	0.001

Disgust 1 > 4: Brain regions in which activation in response to facial expressions of disgust presented first is greater than activation in response to facial expressions of digust preceeded by facial expressions of anger, fear and sadness.
Disgust 4 > 1: Brain regions in which activation in response to facial expressions of disgust preceeded by facial expressions of anger, fear and sadness is greater than activation in response to facial expressions of disgust presented first.

4.5.6 Discussion

In this study I examined the variability of the pattern of neural response to facial expressions of disgust. A reduction of amygdala activation in response to fearful facial expressions has been demonstrated in previous studies (Breiter et al., 1996; Morris et al., 1998; Phillips et al., 2001; Wright et al., 2001). No study to date has shown this effect for disgust, i.e. a decrease in activation over time in the insular response to disgust.

This study replicates previous findings (Phillips et al., 1997, 1998b; Sprengelmeyer et al., 1998) demonstrating the importance of the insula and basal ganglia in the perception of facial expressions of disgust. I have also demonstrated a significant change over time of the pattern of neural response to facial expressions of disgust. This is unlikely to be due to a substantial reduction in attention to the facial stimuli, since the scores on the sex discrimination task in all experiments remained between 92.5 and 100%. My

findings indicate that neural responses to repeated presentations of emotional stimuli do not remain constant.

Activation in the insula did not differ between experiments 1 and 2. The inferior frontal gyrus (BA 45) was activated to a significantly greater extent in experiment 2 compared with experiment 1. Findings from recent studies have indicated that frontal regions may be associated with appraisal of an emotional stimulus rather than the subjective experience of the emotion (Critchley et al., 2000; Lange et al., 2003). Hariri et al. (2000) found amygdala activation when matching facial expressions of fear or anger, but right prefrontal activation when labelling the same facial expressions. Another study (Lane et al., 1997a) demonstrated that attention to subjective emotional responses while viewing emotional picture sets was associated with increased neural activity in the anterior cingulate gyrus.

In the third experiment all observed activation occurred in response to neutral faces. A similar finding has been reported in a study by Phillips et al. (2001), demonstrating a relative increase in activation in the amygdala in response to neutral faces presented in alternation with fearful faces during a 5-minute experiment. This finding has been interpreted as habituation of the amygdala response to fear over the course of the experiment. The similar finding of a pattern of neural response to neutral rather than disgust facial expressions in Experiment 3 may therefore be interpreted as habituation of the insular response to disgust during repeated presentations of facial expressions of disgust. In both studies the relative increase in activation in response to neutral faces over time could be due either to an increase in activation in response to neutral faces or to a decrease in activation (deactivation) in response to facial expressions of disgust. If there were a genuine increase in activation to neutral faces, this might reflect a process of Pavlovian conditioning, in which the disgust expressions act as unconditioned stimuli and the neutral faces (which regularly precede the disgust faces) become conditioned signals of disgust.

In the final experiment the only region of activation in response to disgust faces occurred in the hippocampus, and to a significantly greater extent in experiment 4 than experiment 1. A previous study demonstrated hippocampal involvement in primary visual processing of the face (Kapur et al., 1995). However, a recent study (Fischer et

al., 2003) demonstrating hippocampal activation in response to both neutral and fearful faces showed habituation in the hippocampus over a 2 min. experiment, and the habituation was especially apparent in the right hippocampus. It is possible that no initial hippocampal activation was observed in my data as I have used neutral faces as a control stimulus, whereas the Fisher et al. study used a fixation cross as a control stimulus. If there is hippocampal activation in response to faces per se, then this would not be evident in my data analysis as it would be cancelled out.

It has also been proposed (Gray, 1982; Gray & McNaughton, 2000) that the hippocampus has a role as a comparator, i.e. that it computes the degree to which a stimulus matches, in a context-dependent manner, a template based upon previous regularities of experience. Applying this concept to my study, one may speculate that in experiment 1 the template is set up, in experiment 2 and 3 the template is further strengthened, and in experiment 4, the stimulus is matched to the template. It is possible, therefore, that in experiment 1, the emotional content and perception of the emotion (represented for disgust in the insula) are significant, but that by the fourth experiment, when a subject has been exposed to the stimuli repeatedly, the contextual cuing of the stimulus (represented in the hippocampus) becomes more important than its content.

One explanation for the reduction in magnitude of the neural response, and particularly within the insula, to facial expressions of disgust over the course of the four experiments is habituation of the response within the specialised neural system for the perception of disgust to repeated presentation of these stimuli. Habituation is often described as the simplest form of non-associative learning. It is a decrease in response following repeated stimulus presentation. Habituation has been extensively investigated in both animals (Pavlov, 1927) and humans (Sokolov, 1963). An animal or person first responds to a new stimulus by attending to it. If this stimulus does not have any positive or negative reinforcing qualities the animal or person learns after repeated exposure to ignore it. Sokolov proposed a comparator theory of habituation of the orienting response. The basic proposition is that sensory input is compared with a neuronal model of expected stimulation. When this comparison process results in a match, the orienting response is prevented. When the comparison yields a mismatch, an orienting response is elicited. During the first experiment the faces showing expressions of disgust were new

stimuli that prompted a response, but after repeated presentation, these stimuli would lose their significance. The hippocampus was proposed as fulfilling this role first by Vinogradova (1975) and then by Gray (1982).

The between-group comparisons yielded interesting results. As expected there was no statistically significant difference in activation between experiment 4 in the two groups (one preceded by 3 experiments presenting facial expressions of disgust, the other preceded by 3 experiments presenting facial expressions of anger, fear and sadness). There were, however, differences in activation when comparing the results of experiment 4 of section 4.4 to experiment 1 of section 4.5. Surprisingly there was no statistically significant difference between the two groups in the insula, despite insula activation being present only in the generic brain activation map in experiment 1 of section 4.5. A possible explanation could be sub-threshold activation of the anterior insula in experiment 4 of section 4.4. However, when decreasing the threshold of the generic brain activation map from 50 error pixels to 100 error pixels there was still no insula activation. Therefore it is unlikely that the lack of statistical difference between anterior insula activation in experiment 4 of section 4.4 and experiment 1 of section 4.5 is due to sub-threshold activation of the anterior insula in experiment 4 of section 4.4.

This study has demonstrated that changes in neural responses to facial expressions of disgust occur with repeated presentation of these stimuli. In particular, I report a significant decrease in the normal insular response to expressions of disgust after presentation of these expressions in four successive experiments. My results demonstrate that responses in neural systems important for emotion processing are not fixed but are variable over time. In evolutionary terms, an emotion is an adaptive response to a significant change in the environment, which may be signalled by the facial expressions of others. The significance of the change in the environment will, however, diminish with repeated occurrence. It is therefore important that the neural system important for identification of this change should adapt accordingly, so that the emotion and behaviour generated are contextually appropriate. This finding has important implications for the design of future studies investigating neural responses to emotional stimuli.

4.6 Summary

In this chapter I included 4 levels of data analysis and new experiments to examine the effects that both context, the presence of different emotions, and order, the number of experiments performed, can have on the results of functional neuroimaging studies of emotion.

Firstly, I divided the data set from chapter 3 into 2 groups for each emotion according to whether an emotion was presented first or whether it was preceeded by another emotion. This produced very small group sizes (N = 2-6) and no conclusions could be drawn from these data. Secondly, I added data from a previously published study (Phillips et al., 1998) employing the same experimental design to my data and repeated the analysis. This still yielded small group sizes for facial expressions presented first (N = 3 for fear, N = 5 for disgust) but larger group sizes for those emotions presented as experiment 2-4. When facial expressions of disgust were preceded by an experiment presenting either visual or auditory stimuli of another emotion (anger, fear or sadness) instead of the expected insula activation, activation of the amygdala was demonstrated. Conversely, when facial expressions of fear were preceded by an experiment presenting either visual or auditory stimuli of another emotion (anger, disgust or sadness), activation of the insula instead of the expected amygdala activation was demonstrated. This demonstrates that the context in which facial expressions of a certain emotion are presented can influence the results. Thirdly, I performed an study to confirm those context effects. When facial expressions of disgust were presented in experiment 4 having been preceeded by facial expressions of anger, fear and sadness in experiments 1-3, no insula activation was observed. However, it is not entirely clear whether this is due to the presence of different emotions or simply due to the number of experiments performed, and that activation attenuates regardless of what is presented before experiment 4. It would be interesting to perform another study using only 2 experiments, and investigating what effect different emotions presented first have on the results for facial expressions of disgust presented in experiment 2. Fourthly, in order to investigate whether the number of experiments and the presentation order affect the outcome I repeatedly presented facial expressions of disgust. Again, insula activation was abolished in experiments 3 and 4, though this activation was observed (confirming previous results) in experiments 1 and 2.

My findings confirm that the order of experiment presentation seems to have an effect upon insula activation in response to facial expressions of disgust. It is unclear from the present results whether the context also plays a role, as it is impossible to determine whether the lack of insula activation in experiment 4 of section 4.4 is due simply to repeated presentations of faces as in section 4.5, or specifically due to the fact that the preceding stimuli showed facial expressions other than disgust. Regardless, these results should be taken into consideration when designing further studies.

Chapter 5

Neural Correlates of Olfaction

5.1 Introduction

The neural correlates of olfaction have been investigated in animal, lesion and neuroimaging studies (Zald & Pardo, 2000). The piriform cortex appears to be the primary olfactory cortex and the orbitofrontal cortex, secondary olfactory cortex, with other areas like the amygdala and the insula also believed to play a role in olfaction. The insula receives direct projections from the olfactory system (Carmichael et al., 1994) and is therefore well positioned to be involved in olfactory processes.

It has been shown that unilateral and bilateral orbitofrontal or temporal cortex lesions cause impairments in the discrimination and identification of odours (Jones-Gotman & Zatorre, 1988, 1993; Rausch & Serafetinides, 1975; Zatorre & Jones-Gotman, 1991) and odour memory (Rausch et al., 1977), but it is not known whether such lesions influence the affective ratings of odours. Although several neuroimaging studies have examined the neural correlates of olfaction per se (Koizuka et al., 1994; Royet et al., 2000; Savic et al., 2000; Sobel et al., 1998, 2000; Zatorre et al., 1992), few have investigated the neural correlates of the perception of pleasant and unpleasant odours, with results as yet inconclusive.

The majority of studies employed pleasant olfactory stimuli (Francis et al., 1999; Fulbright et al., 1998; Savic et al., 2000; Zatorre et al., 1992), some employed aversive stimuli (Birbaumer et al., 1998; Zald & Pardo, 1997, 2000), and some examined the neural correlates of both pleasant and unpleasant odours (Anderson et al., 2003b; Fulbright et al., 1998; Royet et al., 2000).

It has been suggested that the amygdala participates in the hedonic processing of olfactory stimuli, especially aversive olfactory stimuli (Zald & Pardo, 2000), as the amygdala receives input from the piriform cortex (Carmichael et al., 1994) and has been activated during exposure to unpleasant stimuli in other sensory modalities (Irwin et al., 1996; Lane et al., 1997; Phillips et al., 1998b; Zald et al., 1998). The orbitofrontal cortex, which is thought to be involved in reward processing per se (Rolls, 1999), and

has been implicated in affective processing in general in previous neuroimaging studies (Davidson & Irwin, 1999; O'Doherty et al., 2001; Zald & Pardo, 1997), is also thought to play a role in the processing of the affective component of odours (Anderson et al., 2003b; Royet et al., 2000, 2001; Zald & Pardo, 1997). This link between reward and the processing of the affective component of odours in the orbitofrontal cortex has been shown very elegantly in an fMRI study investigating sensory specific satiety (O'Doherty et al., 2000). In this study subjects were scanned during exposure to two pleasant odours, vanillin and banana, before eating bananas to satiety. The fMRI scan was then repeated, and while activation to vanillin remained stable in the orbitofrontal cortex, the activation in response to the banana odour decreased, demonstrating that as the banana odour loses its reward value and its pleasantness the activation pattern in the orbitofrontal cortex changes (O'Doherty et al., 2000).

Another region that has been consistently activated in most functional neuroimaging studies of olfaction is the insula. The studies employing pleasant olfactory stimuli reported either bilateral (Francis et al., 1999; Fulbright et al., 1998; Zatorre et al., 1992) or left anterior insula activation (Small et al., 1997a) in response to pleasant odours. Zald and Pardo (2000) reported left anterior insula activation in response to highly aversive odours, but not in response to mildly aversive odorants. Fulbright's study (1998) specifically examined the neural correlates of pleasant and unpleasant odours and reported right insula activation in response to unpleasant odours. Insula activation was also reported in response to acetone (Savic et al., 2002).

Odours have also been shown to differentially modulate the startle reflex in humans, with unpleasant odours enhancing the startle reflex amplitude and pleasant odours reducing the amplitude (Kaviani et al., 1998; Miltner et al., 1994). Odorants have also been shown to induce basic emotions and elicit changes in autonomic nervous system activity, measured by various skin responses, respiratory frequency and heart rate (Alaoui-Ismaili et al., 1997; Vernet-Maury et al., 1999). This suggests a strong hedonic component of odours.

The olfactory system of the human brain is in close association with the limbic system, with potential areas of overlap between olfactory and emotion processing in the insula, amygdala and the orbitofrontal cortex. So far most neuroimaging studies of olfaction

have employed pleasant odours and no distinction has been made between unpleasant and disgusting odours. Previous lesion and neuroimaging studies (Calder et al., 2000; Phillips et al., 1997, 1998b; Sprengelmeyer et al., 1998) have shown the importance of the anterior insula for the perception of disgust in the visual and auditory modality. The anterior insula also appears to be involved in the perception of odours, as has been shown in numerous functional neuroimaging studies (Bengtsson et al., 2001; Francis et al., 1999; Fulbright et al., 1998; Savic et al., 2000; Small et al., 1997a; Zald & Pardo, 2000; Zatorre et al., 1992). A patient who is impaired in the perception of visual and auditory stimuli of disgust (Calder et al., 2000) does not display any olfactory deficit and performs normally on the University of Pennsylvania Smell Identification Test (Doty et al., 1984). His normal olfactory function could be explained by the fact that the lesion is only unilateral, and that his primary and secondary olfactory cortex remain intact.

I hypothesize that the insula is involved in the perception of disgust regardless of the sensory modality of stimulus presentation. As the insula is part of the olfactory system per se I expected to see anterior insula activation in response to all olfactory stimuli used in this experiment, and further expected differential insula activation in response to different odour categories (pleasant, unpleasant and disgusting) with increased activation in the anterior insula in response to disgusting odours. As previous studies have also shown a role for the basal ganglia/ventral striatum in the perception of disgust (Calder et al., 2001; Phan et al., 2002; Phillips et al., 1998b) I also expected to observe activation in response to disgusting odours in this neural region.

5.2 Methods

5.2.1 Subjects

A total of 16 male, right-handed, non-smoking volunteers participated in this experiment. Male subjects were chosen to keep the subject groups similar to the other experiments performed in this thesis, but also because there is some dispute whether there is a difference in brain activation in response to odours in men and women, with some studies reporting a difference (Henkin & Levy, 2001; Yousem et al., 1999) and another reporting no differences (Bengtsson et al., 2001). However, when examining their results closely it becomes apparent that although the activation pattern between

males and females is very similar, there are subtle differences, such as parahippocampal gyrus activation which was observed only in male subjects. Previous studies have mostly used either male (Royet et al., 1999, 2001; Yousem et al., 1997) or female (Savic et al., 2000) subjects, but only few studies have used a mixed subject group (Anderson et al., 2003b; Gottfried et al., 2002) when not specifically examining sex differences in odour perception.

Right-handedness was established using the EHI (see section 3.2.1 for details about the EHI). Non-smokers were chosen as smoking has been shown to influence odour perception (Ahlstroem et al., 1987) and it might also have an effect on brain haemodynamics and hence potentially influence neuroimaging results (Dager & Friedman, 2000). The participants were divided into two groups of 8 (see table 5.2.1 for age, time in full-time education and NART IQ estimate). The two groups were homogeneous as regards age, time in full-time education since the age of 5 and NART (Mann-Whitney U test, $p < 0.05$). All volunteers performed normally on the University of Pennsylvania Smell Identification Test (UPSIT) (Doty et al., 1984). The UPSIT was developed to assess smell function. It consists of a 40-item scratch-and-sniff test. The stimuli are embedded in microencapsules in a strip positioned at the bottom of each test page. The odours are released by scratching the strip with a pencil. The test is forced-choice: above the odorant strip is a multiple-choice question with four possible responses. Test scores are transformed into percentile values according to age and sex of the volunteer using a table provided with the UPSIT. The UPSIT reliably detects microsmia and anosmia. Exclusion criteria also included history of brain injury and past and current psychiatric and neurological illness. All subjects gave informed written consent.

Table 5.2.1: Subject details for group 1 and group 2
All information is given as mean (± STD).

	Group 1	Group 2
Age	27.3 (± 5.1)	30.4 (± 4.8)
Time in Full-time education	17 (± 1.8)	18.5 (± 1.8)
NART IQ estimate	118.9 (± 6)	121.2 (± 5.3)

5.2.2 Stimuli

The stimuli used were banana, vanilla, AR300 (acrid rubbish), SK200 (animal faeces), CV900 (cat urine), IBQ (musty) and fresh air as neutral. All stimuli were supplied by Caravansons Ltd. All odours were diluted in dipropylene glycol (DPG) to the following concentrations: AR300 10%, SK200 50%, CV900 80%, IBQ 60%, banana 12.5%, vanillin 40%. DPG was chosen as it is an odourless solvent. For fresh air the airflow was directed through water. All odorous solutions were mixed a maximum of one week prior to the scan. Fresh water was replaced just before the scan (or before a series of scans on the same day). Vanilla is an odour which is purely olfactory and does not contain a trigeminal component. As the unpleasant odours used do contain a trigeminal component, banana was chosen as a second pleasant stimulus as this also contains a trigeminal component. A study investigating the neural correlates of a purely olfactory odour without any trigeminal component, such as vanilla, compared to odours with mixed olfactory and trigeminal components (Bengtsson et al., 2001) found similar neural circuits in response to both categories of odours, with slightly increased activation in response to odours containing a trigeminal component. In addition to amygdala, piriform and insular cortex activation in response to vanilla, the putamen and caudate were recruited in response to mildly trigeminal odours (Bengtsson et al., 2001). Another study (Yousem et al., 1997) investigating pure olfactory and mixed olfactory and trigeminal odours reported bilateral orbitofrontal activation in response to pure olfactory odours, and a spreading of activation to bilateral primary visual cortex, premotor areas, and the right parietal lobe in response to mixed olfactory and trigeminal odours. However, one study reported different neural circuits for 'pure' odours and odours with a trigeminal component (Savic et al., 2002), observing strong activation of amygdala and piriform cortex in response to vanilla but only very slightly in response to acetone, and activation of thalamus, insular cortex, anterior cingulate and somatosensory cortex in response to acetone. As all the odour categories used in this thesis contained some trigeminal activation, I assume that the main difference is in their affective component rather than a difference in trigeminal component.

All subjects rated the stimuli used during fMRI several days before their arranged scan time to ensure they perceived the pleasant stimuli as pleasant, the disgusting ones as disgusting, and the unpleasant stimuli as unpleasant. Subjects were asked to rate the

stimulus category (pleasant edible, pleasant floral, disgusting, unpleasant but not disgusting, or neutral). Subjects were asked again immediately prior to the scan, when they were already in the scanner, to confirm the category ratings. They were exposed to the odours again and asked to rate them as pleasant edible, pleasant floral, disgusting, unpleasant but not disgusting, or neutral. As odour perception is very subjective, only subjects who rated the odours appropriately for this study (who rated pleasant odours as pleasant, disgusting ones as disgusting, etc.) were included in the fMRI study. For the pre-scan rating the stimuli were delivered through the same facemask (see figure 5.2.3b) as used in the MRI scanner.

5.2.2.1 Stimulus selection

Ten male subjects (mean age 30.4 years, 6 non-smokers) rated the category, pleasantness, intensity and familiarity of 16 odours (6 unpleasant and 10 pleasant). Male subjects were chosen for the ratings to avoid the possible confounds of sex differences in the rating of odours. From the 16 odours 6 were chosen for presentation during the fMRI scan. The criteria for selection were either very high or very low pleasantness ratings and clear category ratings. The odours chosen for the olfactory fMRI were vanillin and banana (pleasant edible), SK200 (animal faeces) and AR300 (acrid rubbish) (disgusting), and CV900 (cat urine) and IBQ (musty) (unpleasant). Tables 5.2.2.1a and 5.2.2.1b detail the ratings of all the odours.

Table 5.2.2.1a: Showing valence, intensity and familiarity ratings for olfactory stimuli

	Valence	Intensity	Familiarity
SK200	–2.9	3.1	-0.9
AR300	–2.3	1.3	–1.1
CV900	–1.5	0.5	–1
IBQ	–1.3	0.8	–1.6
DB100	–1	1.2	-0.9
PCA	–0.9	–1.2	–2.6
Apple	3.2	0.1	2.1
Strawberry	2.7	1.9	3.3
Lily of the Valley	2.5	1.4	4.5
Vanillin	2.5	0.4	2
Banana	2.5	1	2
Chocolate	2.2	0.4	1.6
Lemon Bouquet	2.2	1.8	3.6
Magnolia	2.1	2.4	2.4
Lavender	1.7	2.8	2.8
Crème Caramel	1.7	1.1	1.3

-5 very unpleasant to 5 very pleasant
-5 very weak to 5 very intensive
-5 very unfamiliar to 5 very familiar

Table 5.2.2.1b: Showing the category ratings for the olfactory stimuli (number of subjects who chose each category)

	Pleasant floral	Pleasant edible	Disgusting	Acrid, un-pleasant (not disgusting)
CV900	2		3	5
DB100 (bad breath)	2	1	1	6
SK200 (faeces)	1		7	2
AR300 (rubbish)			6	4
PCA (sweat)	1	2	1	6
IBQ (musty)		2	1	7
Strawberry	5	5		
Apple	6	4		
Banana	2	8		
Lemon	3	6		1
Crème Caramel	2	8		
Chocolate	1	8		1
Lily of the Valley	10			
Lavender	7			3
Magnolia	9		1	
Vanillin		10		

5.2.3 Olfactometer

The olfactometer used to deliver the odours was purpose-built for this experiment, although a similar olfactometer has been employed in a previous fMRI study of olfaction (Yousem et al., 1997). The olfactometer, which contained glass bottles with odorous liquids, was connected to a compressed air cylinder, and airflow was directed through the appropriate bottle by computer-controlled valves (figure 5.2.3a). These valves were controlled from the MR control room via a filtered connector in the penetration panel to reduce any artefact induced by the scanner. The air was bubbled through the odorous liquid to release the odour; this technique has been used successfully before (Kaviani et al., 1998). Each bottle was connected to an individual Teflon tube; Teflon was chose in order to minimise the permeation of the odours into the tubes. The Teflon tubes from the bottles converged in the connector to the facemask to minimise mingling of odours (figure 5.2.3b). The olfactometer was placed close to the magnet at floor level in a lesser magnetic field in order to reduce the dead space in the tube to a minimum. In addition, the space close to the magnet at floor level is the only area in the magnet room where the magnetic field is small enough to allow reliable operation of the valves, which contained small amounts of metal. The odours were delivered to a facemask at a rate of 1 litre per minute. Odours were extracted from the facemask by a vacuum pump at the same rate to minimise mingling of odours in the facemask. For a schematic of the olfactometer set-up see figure 5.2.3c.

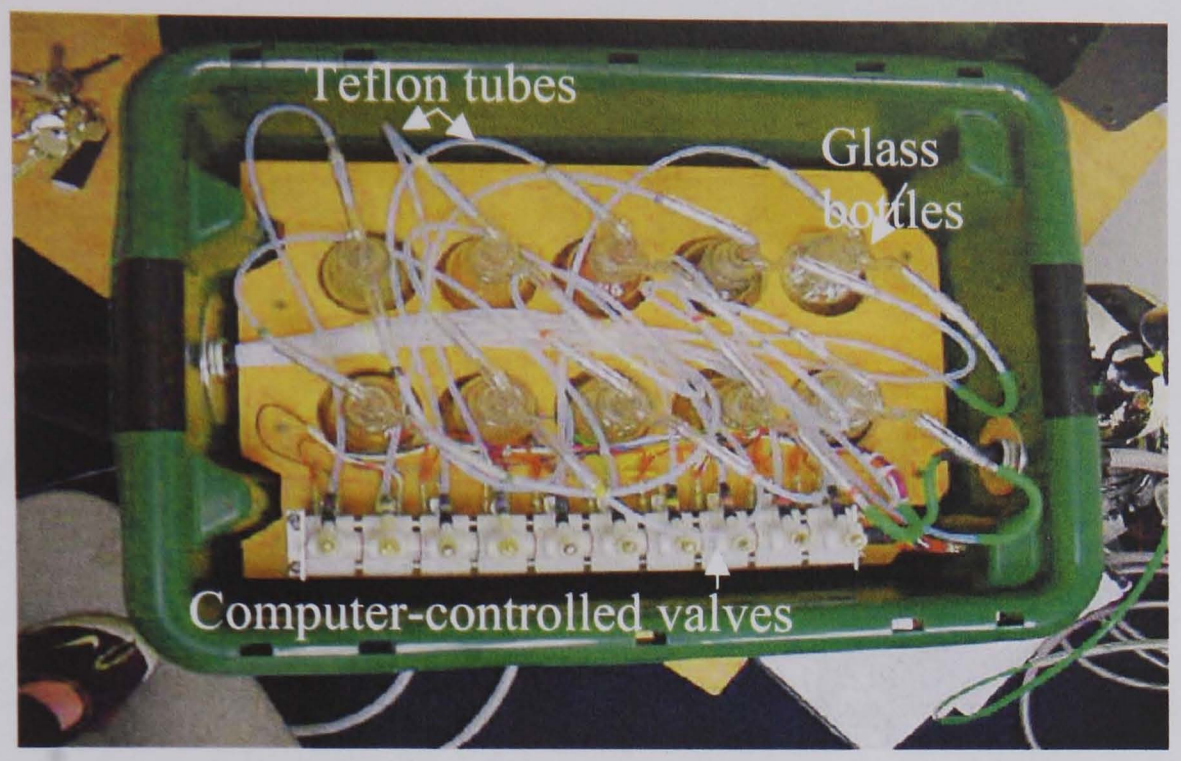


Figure 5.2.3a: Photograph showing the olfactometer.
There are 10 glass bottles containing odorous liquid. Each bottle is connected to the facemask with Teflon tubes to minimize permeation of odours into the tubes. The computer-controlled valves direct the airflow through the selected bottle. One bottle contains fresh water; this is the default option for the airflow.

Figure 5.2.3b: Photograph showing the olfactometer set-up outside the MRI scanner.
Compressed air flows from a cylinder to the olfactometer. The flow rate is set to 1 litre per minute using a flowmeter. The Teflon tubes run inside a big plastic tube for ease of handling to a connector, which is attached to the facemask. The connector is the first place where the Teflon tubes converge.

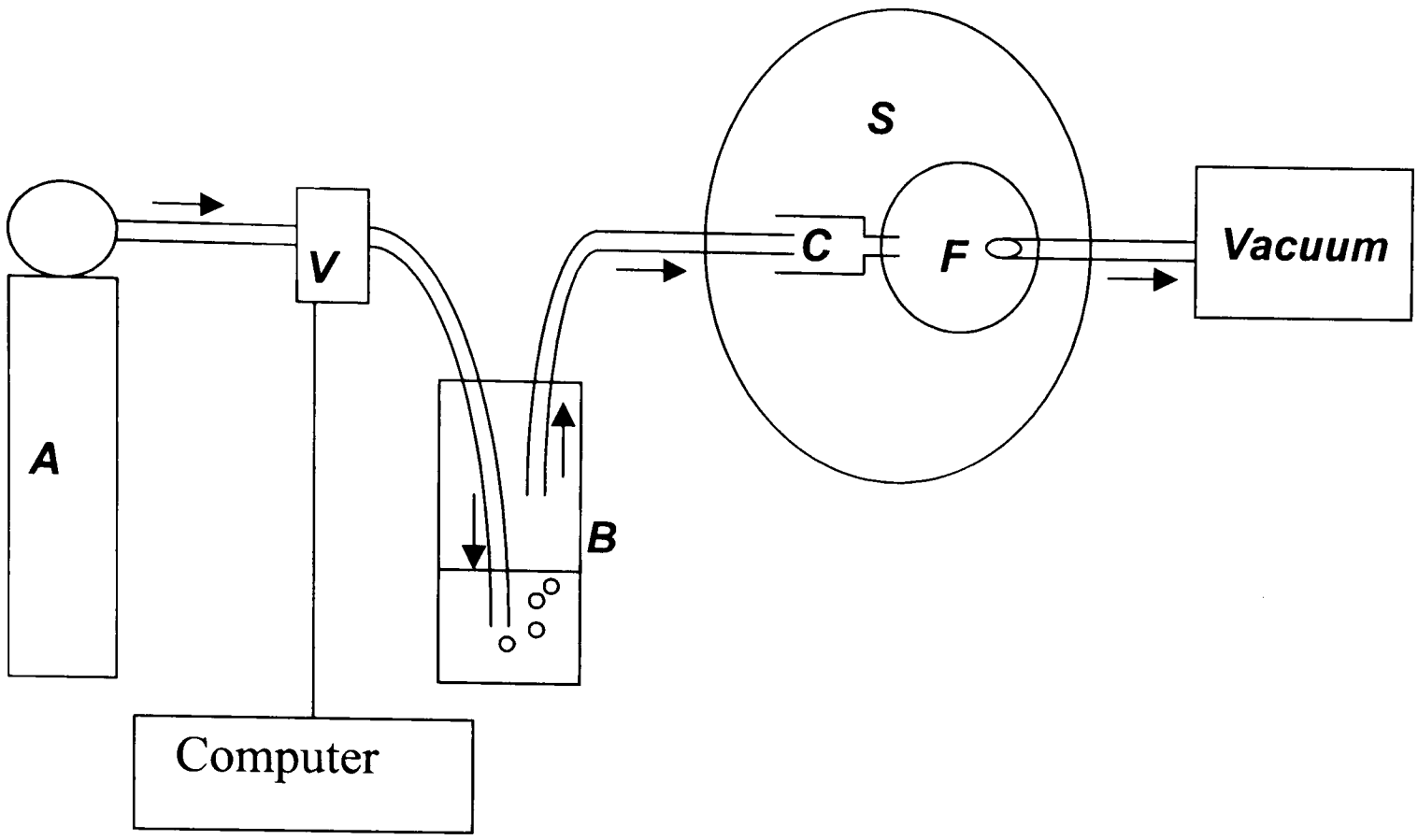


Figure 5.2.3c: Schematic illustrating the olfactometer set-up.
Not to scale. The direction of airflow is indicated by arrows. Air flows into the odorous liquid to release the odour, which is picked up by the air exiting the bottle to the facemask.
A = Air cylinder with regulator and flowmeter
B = Glass bottle containing odorous liquid
C = Connector
F = Facemask
S = MRI scanner
V = Computer-controlled valve

5.2.4 Experimental Design

Two experiments were performed with two separate groups of subjects, one comparing pleasant and disgusting odours (Group 1) to neutral (fresh air), the other comparing unpleasant and disgusting odours (Group 2) to neutral (fresh air). Subjects were asked to eat 2 hours prior to the scan to avoid being hungry and to refrain from caffeine prior to the scan, as caffeine can influence the cerebral blood flow (Dager & Friedman, 2000). In the scanner each subject completed two 5-minute experiments. Each subject was exposed to disgusting (D) odours and either pleasant (P) or unpleasant (U) odours. The order of experiments was P/U D for half of the subjects and D P/U for the other half of the subjects. The 5-minute experiments comprised blocks of 30s ON (alternating 2 odours) and 30s OFF (fresh air) as illustrated in figure 5.2.4. During each ON phase 2 different odours of the same category rating (pleasant, unpleasant or disgusting) were

alternated to prevent habituation. A 6s washout phase was included at the end of every ON phase to avoid any contamination of the fresh air during the neutral phase.

During experiments subjects were instructed to keep their eyes closed and breathe normally.

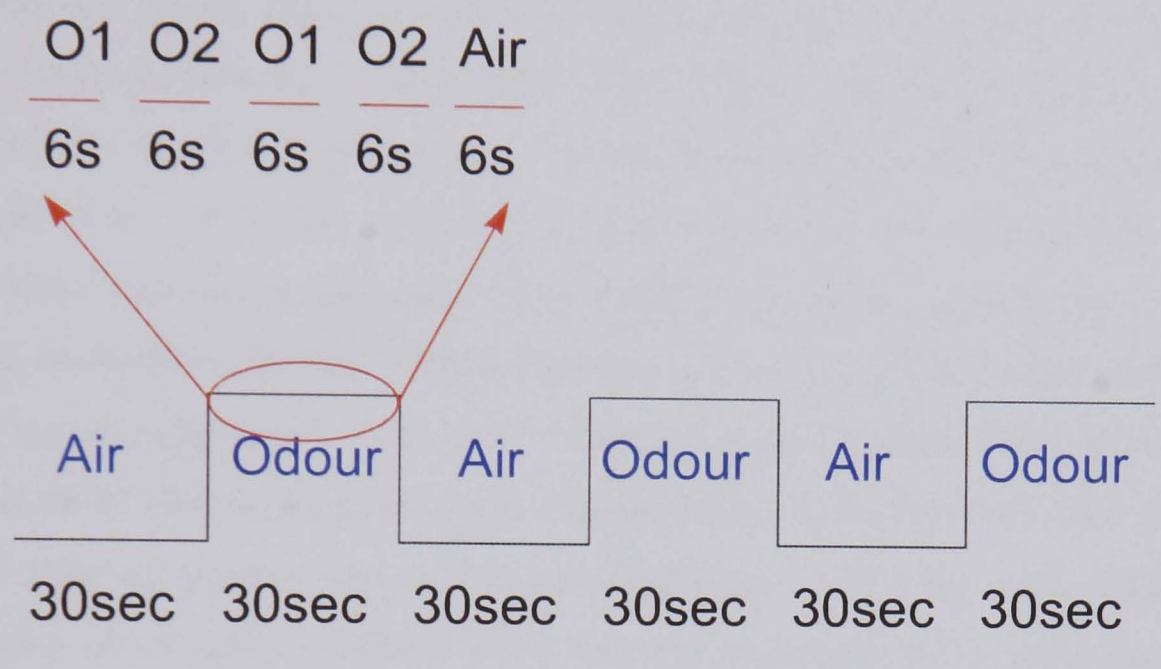


Figure 5.2.4: Design of a 5-minute experiment.
This shows alternating 30 sec blocks of the odour condition (ON) and the neutral fresh air condition (OFF). O1 – odour 1, O2 – odour 2. Each ON phase included a 6 sec washout phase of air at the end the ensure delivery of clean air during the neutral OFF phase.

5.2.5 Image Acqusition

In order to keep the data acquired in the different experiments in this thesis comparable the same acquisition parameters were used as described in section 2.2. This has the disadvantage that the orbitofrontal cortex and the inferior temporal lobes, which are thought to be involved in the hedonic processing of odours, can be affected by signal drop out. Previous fMRI studies of olfaction have used different acquisition planes, special head coils, z-shimming or thin slices to improve the signal from the orbitofrontal cortex and the ventromedial temporal regions (Anderson et al., 2003b; Gottfried et al., 2002; O'Doherty et al., 2000; Sobel et al., 1997). None of these were possible in this experiment as the acquisition parameters had to be kept constant across experiments to allow comparison of results.

5.2.6 Image Analysis

Initial analysis was performed as described in section 2.4.1. As the results were heavily influenced by motion artefacts the data were re-analysed with an updated version of generic brain activation mapping, which had recently become available. The new version has improved capacity for dealing with residual noise in particular (figure 5.2.5a&b), and model fitting is not sinusoidal as in the previous version but uses double gamma functions in order to fit the time-series. Prior to time-series analysis, data were processed to remove low-frequency signal changes and motion-related artefacts (Bullmore et al., 1999). The responses at each voxel were then analysed by regressing the corrected time-series data on a linear model produced by convolving each contrast vector to be studied with two Poisson functions parameterising haemodynamic delays of 4 and 8 seconds (Bullmore et al., 2001). Following least squares fitting of this model, a goodness of fit statistic (Sum of Square Ratio, SSQ) composed of the ratio of model to residual sum of squares was calculated (Edgington, 1995) for each contrast. The distribution of the same statistics under the null hypothesis of no experimental effect was then calculated by wavelet-based resampling of the time-series at each voxel and refitting the models to the resampled data (Bullmore et al., 2001). An experimentally derived null distribution of the goodness of fit statistic was then derived by following this procedure ten times at each intracerebral voxel and combining the resulting data. This method has been shown to give excellent control of nominal type I error rates in fMRI data from a variety of scanners. Activations for any contrast at any required p value can then be determined by obtaining the appropriate critical values from the null distribution (Bullmore et al., 1996). Generic group activation maps were constructed by mapping the observed and randomised test statistics for each individual into the standard stereotactic space of Talairach and Tournoux (1988) and computing and testing median activation maps as previously described (Brammer et al., 1997).

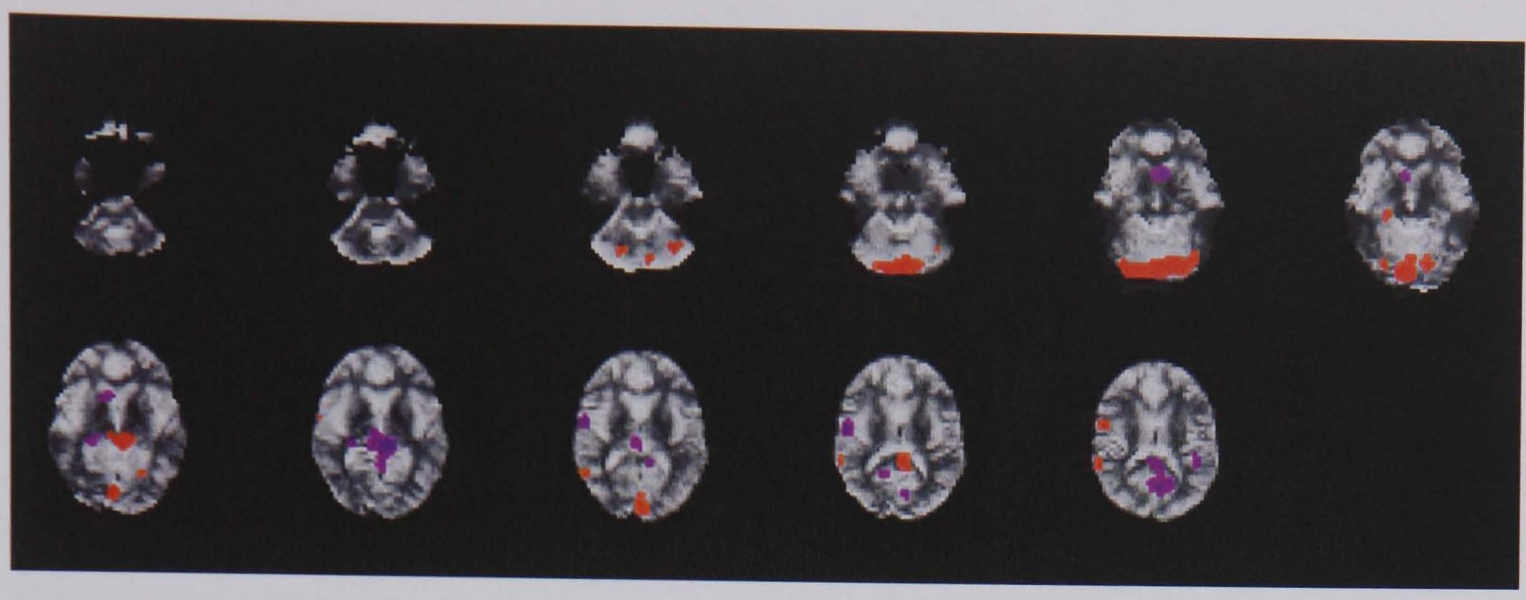


Figure 5.2.5a: Single subject analysis using Generic Brain Activation Mapping

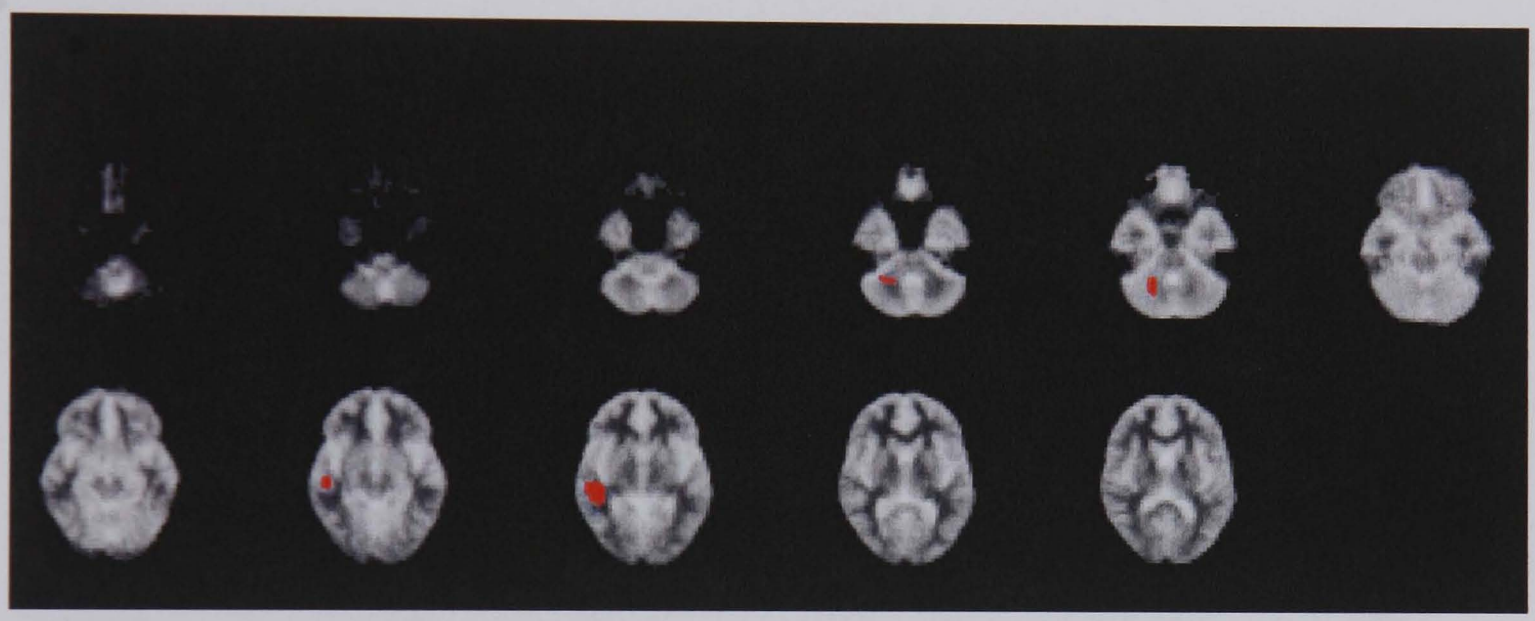


Figure 5.2.5b: Single subject analysis using updated version of Generic Brain Activation Mapping
This figure shows data from the same subject and same experiment as in figure 5.2.5a. The movement induced artefacts, especially in the cerebellum, have been reduced significantly.

I wished to compare directly the intensity of activation within the insula in response to D, P and U odours. The statistic used (SSQratio) is analogous to an F (variance ratio) test without the degrees of freedom in the numerator and denominator. The latter can be difficult to determine in fMRI time-series data. It is used in this context because non-parametric tests make none of the data distributional assumptions and estimates of residual degrees of freedom that underlie an F test. I chose several clusters of activation within the bilateral insula and ventral putamen. Talairach coordinates for Group 1 were $x = 42, y = 20, z = -2$ and $x = -31, y = 25, z = 4$; and for Group 2, $x = 22, y = 10, z = -7$, $x = 35, y = 18, z = -2$ and $x = -36, y = 16, z = 4$. The power of functional response was averaged over each cluster. In order to determine the effect of experimental condition (D vs. P vs. U odours) upon the intensity of activation within each of the chosen

clusters, statistical comparisons of SSQratios in each cluster were made for the two experimental conditions by matched-pairs t-tests. No Bonferroni corrections were made as the data cannot be assumed to be independent.

5.3 Results

5.3.1 Generic brain activation maps

Group 1: Major regions of generic activation in response to disgusting odours

Generic activation was demonstrated in the bilateral anterior insula in response to disgusting odours, with greater activation in the right anterior insula. Further areas of activation include right inferior frontal gyrus (BA 45), right ventrolateral prefrontal gyrus (BA 47) and right posterior cingulate gyrus (BA 31) (figure 5.3.1a & table 5.3.1a).

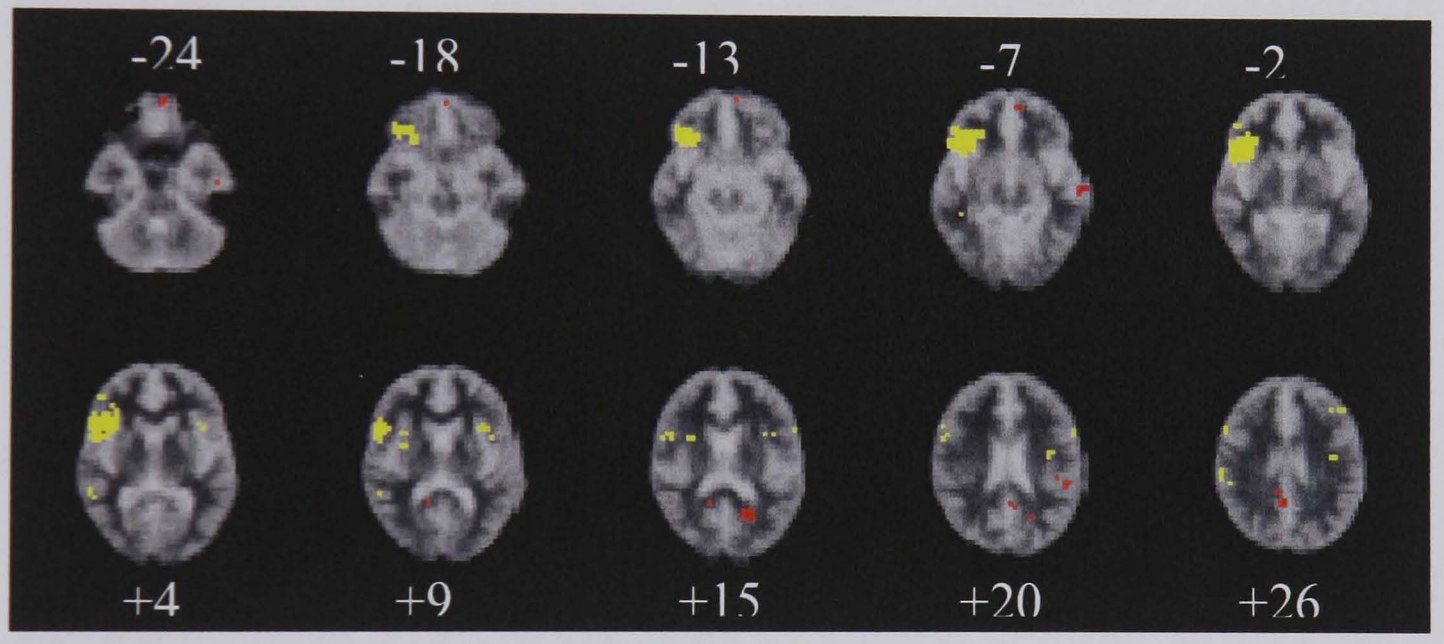


Figure 5.3.1a: Foci of generic brain activation in eight right-handed male subjects during perception of disgusting odours in group 1.
The numbers above and below the transverse sections indicate the distance in mm from the transcallosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by disgusting odours are shown in yellow, and voxels activated at $p<0.004$ during the neutral condition are shown in red.

Group 1: Major regions of generic activation in response to pleasant odours

In response to presentation of pleasant odours, activation in the right posterior cingulate gyrus (BA 31), right ventrolateral prefrontal cortex (BA 47), and left anterior insula (figure 5.3.1b & table 5.3.1b) was observed.

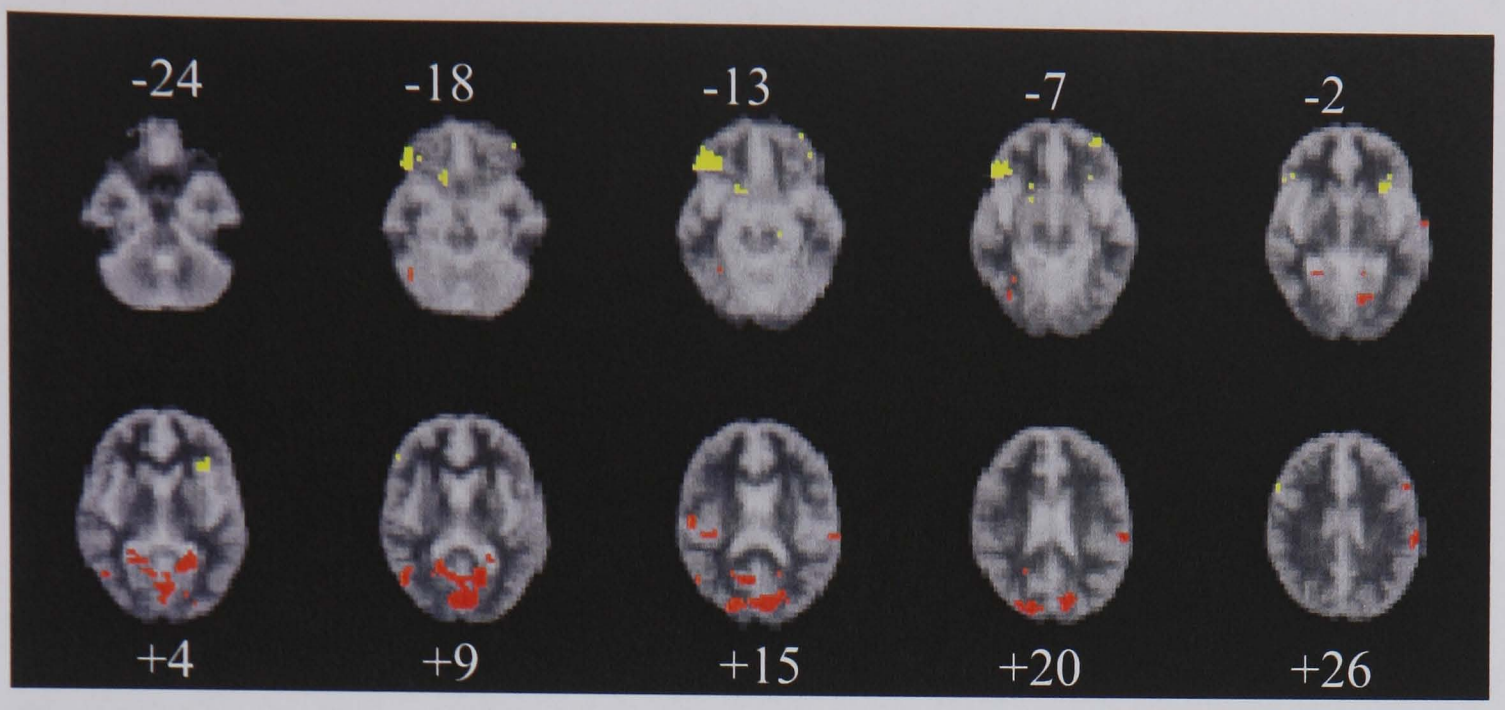


Figure 5.3.1b: Foci of generic brain activation in eight right-handed male subjects during perception of pleasant odours in group 1.
The numbers above and below the transverse sections indicate the distance in mm from the transcallosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by pleasant odours are shown in yellow, and voxels activated at $p<0.004$ during the neutral condition are shown in red.

Group 2: Major regions of generic activation in response to disgusting odours

As in group 1, bilateral anterior insula activation was demonstrated in response to presentation of disgusting odours, with greater activation in the right anterior insula. Generic brain activation was again demonstrated in bilateral ventrolateral prefrontal gyrus (BA 47), right inferior frontal gyrus (BA 44), and right posterior cingulate gyrus (BA 31), in addition to bilateral putamen and right corpus striatum. Both the latter two structures have previously been implicated in the response to facial expressions of disgust (Phillips et al., 1997)(figure 5.3.1c & table 5.3.1c).

Group 2: Major regions of generic activation in response to unpleasant odours

In response to presentation of unpleasant odours, I demonstrated generic activation in the left anterior insula, and left ventrolateral prefrontal cortex (BA 47) (figure 5.3.1d & table 5.3.1d).

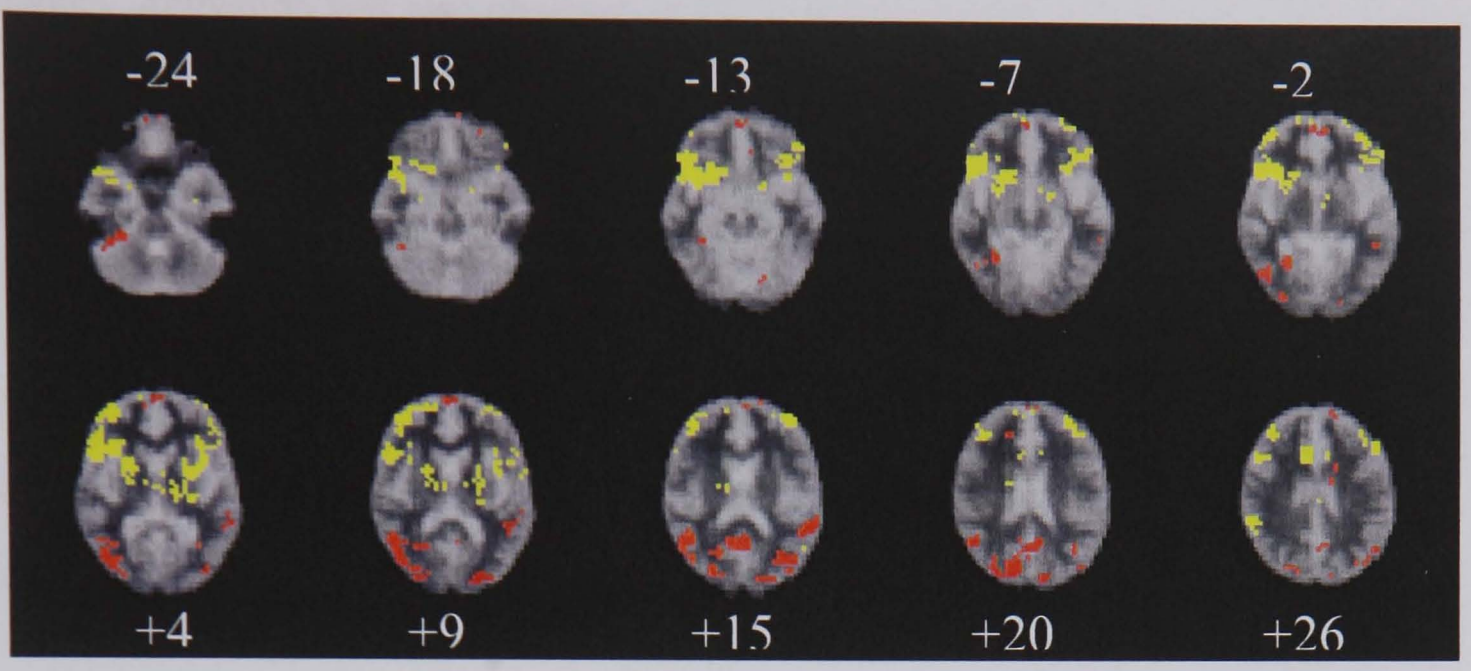


Figure 5.3.1c: Foci of generic brain activation in eight right-handed male subjects during perception of disgusting odours in group 2.
The numbers above and below the transverse sections indicate the distance in mm from the transcallosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by disgusting odours are shown in yellow, and voxels activated at $p<0.004$ during the neutral condition are shown in red.

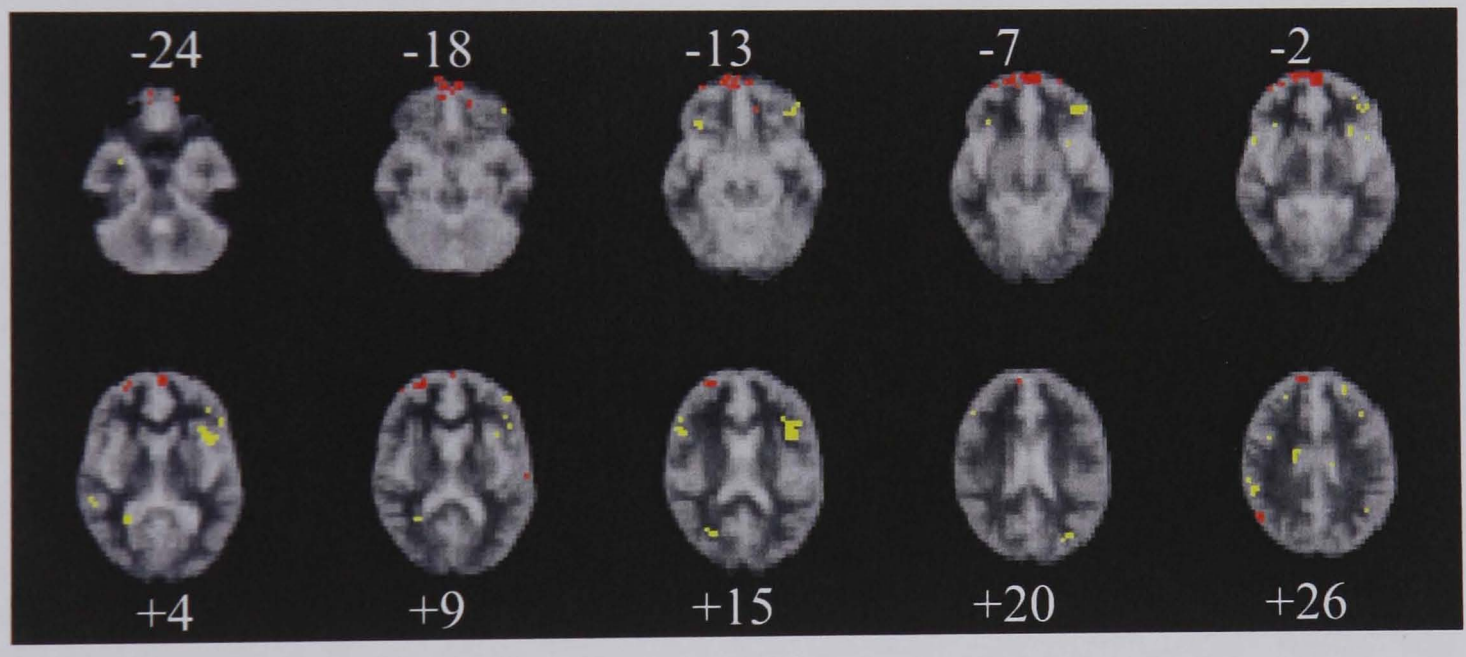


Figure 5.3.1d: Foci of generic brain activation in eight right-handed male subjects during perception of unpleasant odours in group 2.
The numbers above and below the transverse sections indicate the distance in mm from the transcallosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by unpleasant odours are shown in yellow, and voxels activated at $p<0.004$ during the neutral condition are shown in red.

Summary.

Generic brain activation was demonstrated in the left anterior insula in response to all odours contrasted with air, but in the right anterior insula only in response to disgusting

odours. Activation was also observed in the ventrolateral prefrontal cortex (BA 47) in response to all odours, and in right inferior frontal gyrus (BA 44) and posterior cingulate gyrus (BA 31) in response to disgusting and pleasant odours. In Group 2, additional activation was demonstrated within the right ventral putamen in response to disgusting odours.

Table 5.3.1a&b: Main generically activated brain regions in response to pleasant and disgusting odours in group 1.

Cerebral Region (Brodmann Area)	Side	x[a]	y[a]	z[a]	No. of voxels	Condition of signal increase[b]
a) Group 1: Disgusting Odours						
Inferior Frontal Gyrus (45)	R	44	20	4	56	Disgust
Insula	R[c]	42	20	-2	42	Disgust
	L	-32	16	9	4	
Ventrolateral Prefrontal Cortex (47)	R	40	26	-7	38	Disgust
Premotor Cortex & SMA (6)	R	49	14	9	20	Disgust
Posterior Cingulate Gyrus (31)	R	9	-41	26	15	Neutral
b) Group 1: Pleasant Odours						
Ventrolateral Prefrontal Cortex (47)	R	43	26	-13	19	Pleasant
Insula	L[d]	-31	25	4	5	Pleasant
Posterior Cingulate Gyrus (31)	R	1	-69	9	55	Neutral
Primary Visual Cortex (18)	L	-1	-73	4	17	Neutral
	R	19	-81	20	11	
Inferior Parietal Lobule (40)	L	-54	-24	31	10	Neutral

[a] The cluster with the largest number of voxels in each region is reported. Talairach coordinates refer to the voxel with the maximum Sum of Square Ratio (SSQratio) in each regional cluster. All activated voxels were identified by a one-tailed test against the null hypothesis that median SSQratio is not determined by experimental design. The probability threshold for activation was $p \leq 0.004$, this means the expected type 1 error rate is 4 false positive voxels per slice.

[b] Signal increase was detected either during presentation of disgusting/unpleasant/pleasant odours or neutral baseline (fresh air) odour.

[c] Significant difference between conditions.

[d] No significant difference between conditions.

Table 5.3.1c&d: Main generically activated brain regions in response to unpleasant and disgusting odours in group 2.

Cerebral Region (Brodmann Area)	Side	x[a]	y[a]	z[a]	No. of voxels	Condition of signal increase[b]
c) Group 2: Disgusting Odours						
Ventrolateral Prefrontal Cortex (47)	R	36	17	-13	53	Disgust
	L	-36	28	-7	17	
Insula	R[d]	38	16	4	50	Disgust
	L[d]	-34	6	9	6	
Putamen	L	-27	4	4	40	Disgust
Medial Frontal Lobe (32)	R	1	18	37	39	Disgust
Anterior Cingulate Gyrus (24)	L	-21	13	31	11	Disgust
Superior Temporal Gyrus (22)	R	45	-56	15	18	Disgust
	L	-51	-42	15	14	
Dorsolateral Prefrontal Cortex (9)	R	43	10	37	33	Disgust
Ventral Putamen	R[c]	22	11	-7	17	Disgust
Premotor Cortex & SMA (6)	R	50	7	9	14	Disgust
Dorsolateral Prefrontal Cortex (46)	R	38	45	15	11	Disgust
Inferior Frontal Gyrus (44)	R	38	49	4	11	Disgust
Posterior Cingulate Gyrus (23/31)	R	13	-69	20	31	Neutral
		5	-55	15	18	
Inferior posterior temporal lobe (37)	R	42	-62	9	23	Neutral
Postcentral Gyrus (2)	L	-37	-27	42	19	Neutral
Supramarginal Gyrus (40)	R	53	-38	31	13	Neutral
	L	-33	-28	37	12	
Angular Gyrus (39)	L	-33	-68	15	11	Neutral
Cerebellum	R	31	-44	-24	10	Neutral
d) Group 2: Unpleasant Odours						
Insula	L[d]	-35	16	4	10	Unpleasant
Ventrolateral Prefrontal Cortex (47)	L	-40	30	-7	5	Unpleasant
Medial Frontal Gyrus (10)	R	1	63	-2	10	Neutral
Orbitofrontal Cortex (11)	R	2	61	-18	10	Neutral

[a] The cluster with the largest number of voxels in each region is reported. Talairach coordinates refer to the voxel with the maximum Sum of Square Ratio (SSQratio) in each regional cluster. All activated voxels were identified by a one-tailed test against the null hypothesis that median SSQratio is not determined by experimental design. The probability threshold for activation was $p \leq 0.004$, this means the expected type 1 error rate is 4 false positive voxels per slice.

[b] Signal increase was detected either during presentation of disgusting/unpleasant/pleasant odours or neutral baseline (fresh air) odour.

[c] Significant difference between conditions.

[d] No significant difference between conditions.

5.3.2 Within-group comparison of activation in anterior insula and putamen

In Group 1, the comparison of a measure of the mean intensity of activation within the anterior insula and ventral putamen across conditions revealed a significant difference in response to disgusting compared with pleasant odours in the right anterior insula (Talairach coordinates of the centre of the cluster: x = 42, y = 20, z = -2,) at p = 0.05 (figure 5.3.2). No difference in activation was found in the left anterior insula (Talairach coordinates for the centre of the cluster: x = -31, y = 25, z = 4). In Group 2, there was a significant difference in BOLD signal change in response to disgusting compared with unpleasant odours in the right ventral putamen (Talairach coordinates of the centre of the cluster: x = 22, y = 10, z = -7) at p = 0.01 (figure 5.3.2). No statistically significant difference in activation was found in the (a) right or (b) left anterior insula (Talairach coordinates of the centres of these clusters: (a) x=35, y=18, z=-2, (b) x=-36, y=16, z=4).

5.3.3 Between-group comparison of generic brain activation

The generic brain activation in response to disgusting odours in both groups was compared in order to establish whether there were any differences between the groups. However, no statistically significant difference in activation was found between the two groups in response to disgusting odours.

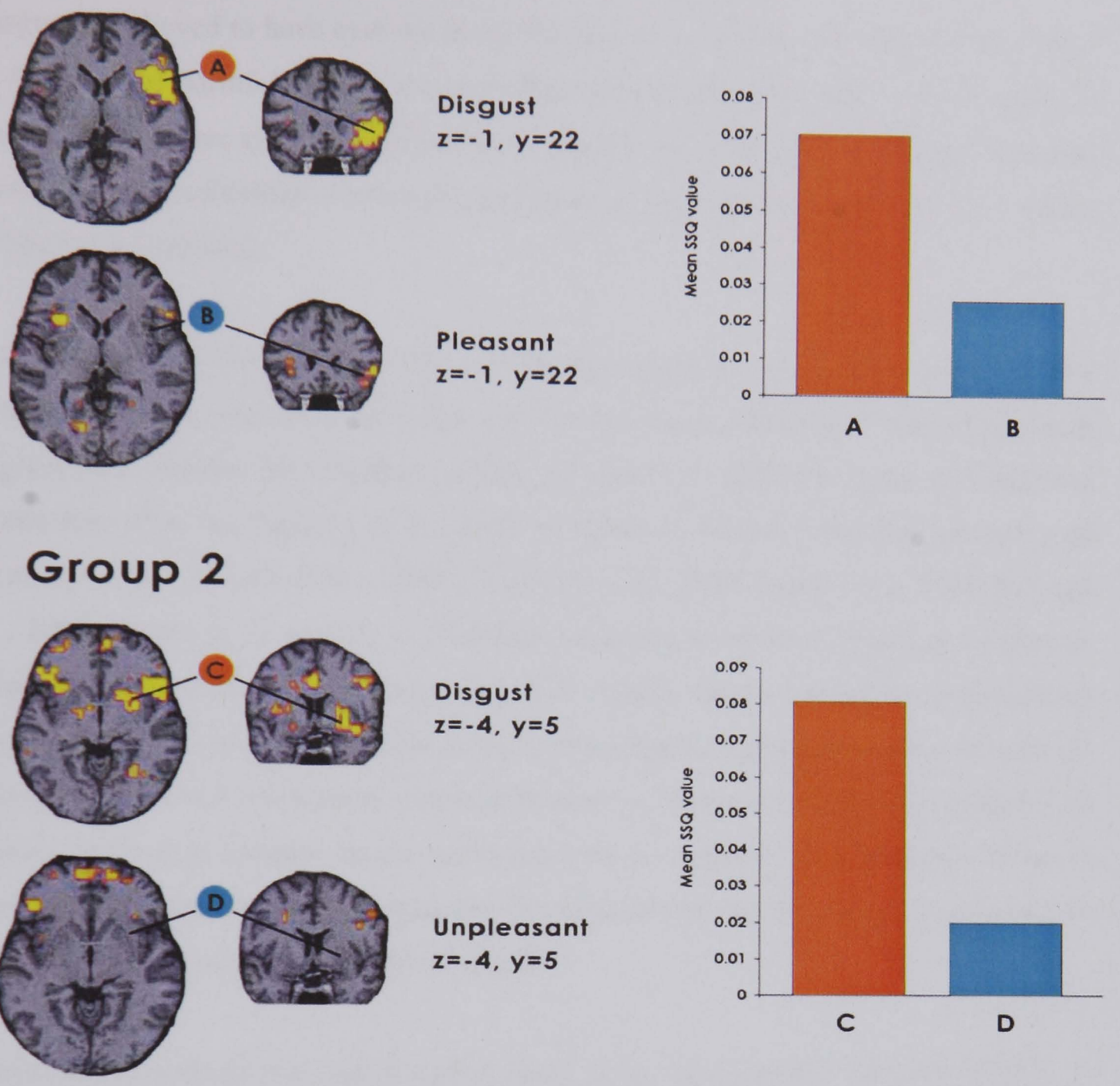


Figure 5.3.2: Within-group comparison of activation
Major clusters of generic brain activation in response to disgusting and pleasant (Group 1), and disgusting and unpleasant but not disgusting (Group 2) odours compared with air are demonstrated in axial (z = -1, and z = -4 for Groups 1 and 2, respectively) and coronal (y = 22, and y = 5 respectively) brain slices. The right side of the brain is shown on the right side of the figure and the left hemisphere is shown on the left side of the figure. In Group 1, the magnitude of functional response (mean SSQratio) in a cluster within the right anterior insula (cluster centre: x = 42, y = 20, z = -2) was significantly greater (p = 0.05) to disgusting odours compared with air (cluster A) than to pleasant odors compared with air (cluster B). In Group 2, the magnitude of functional response (mean SSQratio) in a cluster within the right ventral putamen (centre x = 22, y = 10, z = -7) was significantly greater (p = 0.01) (cluster C) to disgusting odours compared with air than to unpleasant odours compared with air (cluster D).

5.4 Discussion

Disgust is believed to have evolved as an emotion to protect the individual from danger in the form of harmful substances, including those detected by the sense of smell. A close link between systems specialized to react to harmful odours, whether detected directly by the individual or indirectly by way of conspecific facial expression, is likely to have aided survival.

Previous studies (Phillips et al., 1997, 1998b; Sprengelmeyer et al., 1998) have reported activation of the anterior insula and ventral putamen in response to disgusting facial expressions. Studies investigating neural correlates of olfaction have demonstrated insula activation in response to a variety of olfactory stimuli including pleasant and unpleasant ones (Francis et al., 1999; O'Doherty et al., 2000; Royet et al., 2001; Savic et al., 2000; Small et al., 1997). I therefore expected to observe insula activation in response to all odours. I predicted that the anterior insula would be activated by disgusting odours to a significantly greater extent than by pleasant or other unpleasant odours. I observed left anterior insula activation in response to different categories of odours, with right anterior insula activation only in response to disgusting odours. I therefore speculate that it is specifically the right anterior insula, which is involved in the processing of disgusting olfactory stimuli.

Based on previous neuroimaging studies using facial expressions of disgust (Phillips et al., 1997, 1998, 1999; Sprengelmeyer et al., 1998) I had also predicted ventral striatal activation in response to disgusting odours, as right-sided ventral striatal activation has been reported in all of the above studies. However, I observed activation of the right ventral putamen in response to disgusting odours only in study 2 but not in study 1.

Activation of ventrolateral prefrontal cortex (BA47) was observed in response to all conditions. This structure has been implicated in the processing of emotions per se. Neuroimaging studies have demonstrated increased blood flow in this area in response to facial expressions displaying different negative emotions (Sprengelmeyer et al., 1998), but also during the induction of sadness and guilt and during the recall of emotional material (Phillips, 2003).

I did not find activation in primary (piriform) or secondary (orbitofrontal) olfactory cortex. This could be due to several factors. The acquisition sequence I used in this study is the same as in previous studies (Phillips et al., 1997) to allow comparison of data across studies, and is not ideal for imaging orbitofrontal cortex (Zald & Pardo, 2000). As the acquisition plane in this study was transverse rather than coronal (O'Doherty et al., 2000) or specially tilted (Anderson et al., 2003b; Poellinger et al., 2001; Sobel et al., 1997) as in other fMRI studies of olfaction, there was signal dropout in the orbitofrontal cortex, an example of which is shown in figure 5.4 for several subjects. The slice thickness in this study was 7mm, which can also contribute to signal dropout due to the partial voluming effect caused by air-filled cavities ventral to the orbitofrontal cortex being included in the data acquisition. This effect can be reduced by acquiring thinner slices (Anderson et al., 2003b; Sobel et al., 1997). As the main focus of this work is on the anterior insula cortex and striatal structures rather than on the orbitofrontal cortex, and as I chose the data acquisition sequence to match the other experiments in this thesis, I accepted this disadvantage of the transverse data acquisition.

The primary olfactory cortex in humans (Poellinger et al., 2001; Sobel et al., 2000) has been shown to have very transient and rapidly habituating responses, which means that across a 30-s block of activation as used in this experiment it is impossible to detect the initial response. Furthermore, the piriform cortex is also activated by the mechanical movement of the nasal hair, which occurs during sniffing and smelling (Sobel et al., 1998). This should be the same during the odour condition and the neutral fresh air condition, which makes it even more difficult to detect any additional activation in response to the odour. Other functional neuroimaging studies of olfaction also failed to observe activation of the primary olfactory cortex (O'Doherty et al., 2000; Royet et al., 2001; Sobel et al., 1998; Yousem et al., 1997; Zald & Pardo, 1997), possibly due to the above mentioned reasons as well, although it has been suggested that cognitive features may influence the extent of piriform cortex activation detected in subtraction analyses (Dade et al., 1998; Zald & Pardo, 2000b).

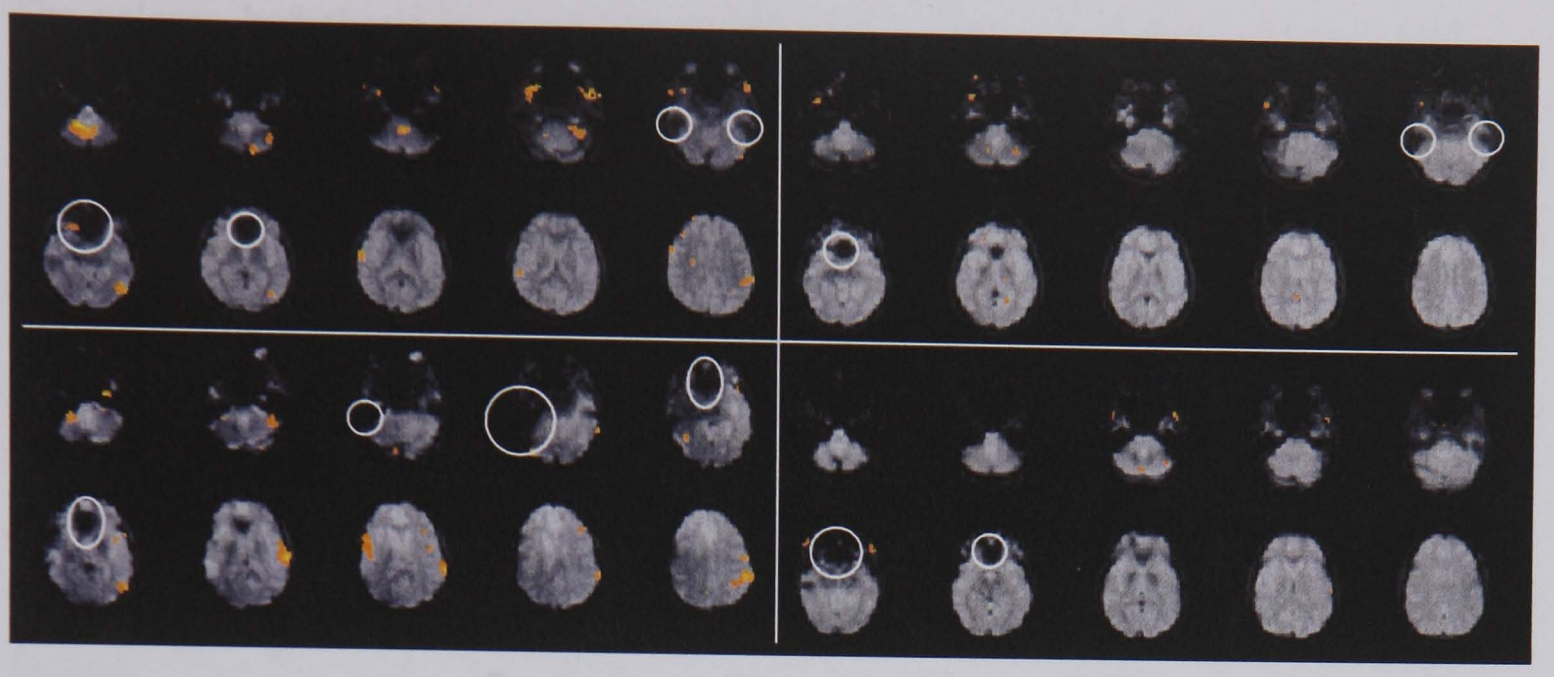


Figure 5.4: Examples of signal dropout
Due to the acquisition parameters used there was some signal dropout in the orbitofrontal and inferior temporal lobes. These are examples of four subjects' individual brain activation maps in response to disgusting odours prior to mapping into Talairach space. Examples of signal dropout in the orbitofrontal cortex and ventral temporal lobes are encircled in white.

There was no difference in left anterior insula activation in the response to disgusting odours compared with that to pleasant odours (study 1), nor in the response to disgusting odours compared with that to unpleasant odours (study 2). This may reflect the role of the left anterior insula in olfactory perception per se. However, I did observe a significant difference in the level of activation of the right anterior insula in response to disgusting odours as compared to pleasant odours (study1), and of the level of activation of the right ventral putamen in response to disgusting odours as compared to unpleasant odours (study 2), as shown in Figure 2. This hemispheric lateralization was unexpected. Previous studies employing visual stimuli of facial expressions of disgust have reported differing results as far as lateralization is concerned, ranging from mainly right anterior insula activation (Phillips et al., 1997) to bilateral activation (Phillips et al., 1998b) to mainly left anterior insula activation (Phillips et al., 1999; Sprengelmeyer et al., 1998). A study employing disgusting pictures (Phillips et al., 2000) reported left anterior insula activation in response to these. The patient who was impaired at recognizing facial and vocal expressions of disgust had a left-sided insular and striatal lesion (Calder et al., 2000). The above-mentioned studies appear to support a role for the left anterior insula in the perception of visual stimuli of disgust, which is contrary to the valence hypothesis of cerebral asymmetry.

The valence hypothesis is grounded in clinical and EEG data (Davidson & Sutton, 1995) and posits that the experience or expression of emotional valence is lateralized across the hemispheres of the brain. Positive (or approach-related) emotions are thought to be lateralized towards the left and negative (or withdrawal-related) emotions towards the right frontotemporal regions of the brain. So far, only one neuroimaging study has directly tested this hypothesis and does support it (Canli et al., 1998). In addition, one fMRI study investigating responses to pleasant and unpleasant odours reported right-sided activation in response to unpleasant odours and left-sided activation in response to pleasant odours (Henkin & Levy, 2001), supporting the valence hypothesis. My findings from this study also suggest a generally more important role of the right hemisphere for the perception of negative emotions. Adequate interpretation of this lateralization of disgust requires evidence as to whether other species display similar phenomena.

However, the lateralization of olfactory function in the anterior insula is not entirely clear. Olfaction is unique among the senses as the olfactory nerve projects ipsilaterally and not contralaterally like the second order neurons in other senses, including the trigeminal system (Brand & Jacquot, 2001). As the odours were presented birhinally in this study and as the insula receives direct input from the primary olfactory cortex (Price, 1987) bilateral activation of the insula was expected in response to all odours. Previous neuroimaging studies of olfaction report different insula activation. The majority of studies report bilateral anterior insula activation in response to passive smelling of odours (Francis et al., 1999; Fulbright et al., 1998; O'Doherty et al., 2000; Poellinger et al., 2001; Zatorre et al., 1992), but some report left insula activation (Savic et al., 2000; Small et al., 1997; Zald & Pardo, 2000).

The activations in response to the neutral stimulus (fresh air) is difficult to interpret, as it is unclear whether these are real activations in response to fresh air, or whether they represent a decrease in blood flow to these areas in response to the odours and therefore appear as a relative increase in blood flow in response to fresh air. One region showing consistent activation in response to fresh air in three out of the four experiments is the posterior cingulate gyrus (table 5.3.1), which has been associated with emotional processing (Maddock, 1999).

One drawback of this study was the lack of behavioural measures during the fMRI experiment, unlike the experiments described in chapters 3 and 4. It is therefore not possible to be certain that subjects stayed awake and paid attention to the odours throughout the experiment. Cognitive tasks as behavioural measures were deliberately excluded here as they can influence results (Royet et al., 2001; Savic et al., 2000), and the main focus of interest in this experiment was the difference in activation in response to odours of different hedonicity. Furthermore, it is possible that the observed difference in activation in response to pleasant, unpleasant and disgusting odours could be due to differences in the intensity or familiarity of the odours used (table 5.2.2.1a). For future experiments it might, therefore, be advantageous to take ratings of the odours immediately after the scan, as has been done in a previous PET study (Savic et al., 2002).

My results support the hypothesis of distinct neural substrates underlying the perception of particular emotions, specifically that the anterior insula and ventral putamen are involved in the perception of disgust. Additionally, my findings together with those from previous studies indicate that the right anterior insula responds to disgust presented in different sensory modalities, both visual and olfactory. The only inconclusive results so far have been in the auditory modality, the only published study (Phillips et al., 1998b) does not report activation in the anterior insula in response to disgusting non-verbal vocal stimuli, whereas the results described in chapter 3 do show activation of the anterior insula in response to auditory stimuli of disgust. As disgust appears to have evolved to enable the avoidance of ingestion of harmful substances, olfaction and gustation would be particularly important senses for the detection of such substances. It is, however, also important to be able to recognize facial expressions of disgust in others to facilitate vicarious learning of the avoidance of these substances in the environment. The findings from studies examining neural responses to auditory presentations of disgust may be a result of the lower probability of encountering an auditory presentation of disgust in the environment than a presentation of disgust in the olfactory, gustatory or visual modality.

My findings demonstrate a specific role of the right anterior insula and ventral putamen in the response to disgusting stimuli presented in the olfactory modality. Given previous findings highlighting the importance of these regions in the response also to visual

displays of disgust, I propose that right-sided anterior insula and ventral putamen are key components in a system mediating the response to disgusting stimuli irrespective of sensory modality.

The next chapter looks at perception of disgust in the gustatory modality.

Chapter 6

Neural Correlates of Gustation

6.1 Introduction

Within the past decade a relatively small number of human neuroimaging studies investigating neural correlates of taste perception have been carried out. Many of these studies have used positron emission tomography and have employed simple experimental protocols to produce taste stimulation. The literature regarding functional neuroimaging of taste perception in humans has been reviewed in section 1.4.4.3. Here I will focus on the different methods that have been employed to deliver taste stimuli to the subjects, and discuss some of the difficulties associated with delivering tastes in an fMRI environment.

A good overview of PET studies investigating human cortical taste areas is given in the review by Small (1999). The first study to investigate neural correlates of taste perception was performed using PET in 1994 (Kinomura et al., 1994). In this study 0.2 ml of either water or saline solution were injected every 15 sec into the subjects' mouth through plastic tubes. It is not described in the methods if this was performed by hand or computer-controlled. A similar method is employed by other studies, injecting solutions into the mouth using a cannula (Zald et al., 1998). Less accurate methods including delivery of drops by pipette, 'painting' solution onto the tongue with a wooden stick, and placing pieces of chocolate (Zald et al., 1998) or filter papers soaked in different tastes into subjects' mouth (Small et al., 1999). The filter paper method has been used by Small et al. in two studies (Small et al., 1997b, a): the filter papers are replaced every 7 sec throughout the experiment. This would not be possible in an fMRI environment, as the experimenter does not have access to the subject's head which is inside the magnetic bore, unlike the PET camera, which still allows easy access to the subject's head. Having subjects opening and closing their mouths every 7 sec would also cause enormous movement artefacts, which would be difficult to control for during data analysis. None of these methods facilitates precise control of the timing of stimulus delivery, which is desirable for fMRI experiments.

Since 1999 most neuroimaging studies examining human gustatory areas have used fMRI, one exception is a study by Small et al. (2001) looking at changes in brain activity in response to eating chocolate. In this PET study pieces of chocolate were directly applied to the tongue. FMRI studies (Faurion et al., 1999; Francis et al., 1999; O'Doherty et al., 2001) and a PET study (Zald et al., 2002) since then have delivered gustatory stimuli through tubes or cannulae, but it is not always clear if the delivery was controlled manually or by computer. The studies used delivery rates of 50 µl every 3 sec (Faurion et al., 1999), 0.5 ml every 8 sec (Francis et al., 1999; O'Doherty et al., 2001), and 3 ml at the start of the scan and a further 2-6 ml over the next 40 sec (Zald et al., 2002).

There are several problems with delivering gustatory stimuli in an fMRI environment. As mentioned above, there is no direct access to the head of the subject, which means the stimulus needs to be delivered over a fairly long distance. It can be difficult to control exact delivery of stimuli over a distance of several meters. If the delivery is to be computer-controlled, the computer will have to be based in the control room, which is where the machine interfacing between computer and tubes containing liquid stimuli also needs to be placed, unless it is possible to build this with minimal metal content. Administering liquid stimuli is going to make the subjects want to swallow, which can cause a movement artefact in the fMRI data. In order to reduce swallowing most studies have administered only small amounts of liquid. Another reason to deliver only small quantities is the potential risk of subjects choking. It is more difficult to swallow in a supine position than when sitting or standing upright, and if somebody were to choke it would be difficult for them to sit up or get out of the MRI scanner immediately. Delivering minute amounts also helps to reduce the olfactory component of flavours, such as peach-flavoured ice tea as used in this study, to ensure that all stimuli are as close to pure tastes (sweet, salty, bitter, sour, umami) as possible.

Previous findings (Francis et al., 1999; O'Doherty et al., 2001; Small et al., 1999) suggest an overlap between regions important for perception of flavours and those involved in emotion perception, namely the insula, amygdala and the orbitofrontal cortex. Disgust is the only emotion that can easily be directly translated from vision and audition into the gustatory modality. Previous studies have investigated the neural correlates of unpleasant or aversive gustatory stimuli but no study to date has directly

investigated the neural response to disgusting tastes. Although one study (Zald et al., 2002) reports subjects' description of an extremely aversive quinine solution as "disgusting", "gross" and "horrible", no direct connections with disgust or the neural correlates of disgust were made.

The aim of this study included the design of a gustometer capable of delivering a number of different taste stimuli to subjects inside an fMRI scanner. Using this gustometer, the neural correlates of disgusting tastes were investigated and compared to the neural responses to pleasant and unpleasant tastes. Based on previous lesion and neuroimaging studies (Calder et al., 2000; Phillips et al., 1997, 1998b; Sprengelmeyer et al., 1998) I hypothesized that the insula is involved in perception of disgust regardless of the sensory modality of stimulus presentation. As the insula is also part of the gustatory system per se, and is even considered primary gustatory cortex (Ogawa, 1994), I expected to observe insula activation in response to all categories (pleasant, unpleasant and disgusting) of taste stimuli, and to observe significantly increased activation in the anterior insula in response to disgusting tastes as compared to pleasant or unpleasant tastes.

6.2 Methods

6.2.1 Subjects

Two groups, each of 8 right-handed, volunteers (6 male, 2 female in each group), participated in this experiment. Handedness has been shown to influence pattern of brain activation in response to tastes (Faurion et al., 1999), and therefore only right-handed subjects were included in the study. Right-handedness was established using the EHI (see section 3.2.1 for details about the EHI). Non-smokers were chosen as smoking might have an effect on brain haemodynamics and hence potentially influence neuroimaging results (Dager & Friedman, 2000). Three subjects participated twice and were included in both group 1 and group 2 (see table 6.2.1 for age, time in full-time education and NART IQ estimate). The two groups were homogeneous as regards age, time in full-time education since the age of 5 and NART (Mann-Whitney U test, $p > 0.05$). Exclusion criteria also included history of brain injury and past and current psychiatric and neurological illness. All subjects gave informed written consent.

Table 6.2.1: Subject details for group 1 and group 2
All information is given as mean (± STD).

	Group 1	Group 2
Age	23.8 (± 5.2)	31.4 (± 7.2)
Time in full-time education	18.2 (± 3.1)	17.6 (± 3.7)
NART IQ estimate	118.5 (± 7.2)	122.4 (± 3.4)

6.2.2 Stimuli

All subjects rated the stimuli to be used during the fMRI experiment several days before their arranged scan time to ensure that they perceived the pleasant stimuli as pleasant, the disgusting ones as disgusting, and the unpleasant stimuli as unpleasant. Subjects were asked to rate the stimulus category. As taste perception is very subjective only subjects rating the tastes appropriately for this study were included in the fMRI study. For the rating the stimuli were delivered through the same gustometer used in the MRI scanner. During experiments subjects were instructed to keep their eyes closed.

6.2.2.1 Stimulus selection

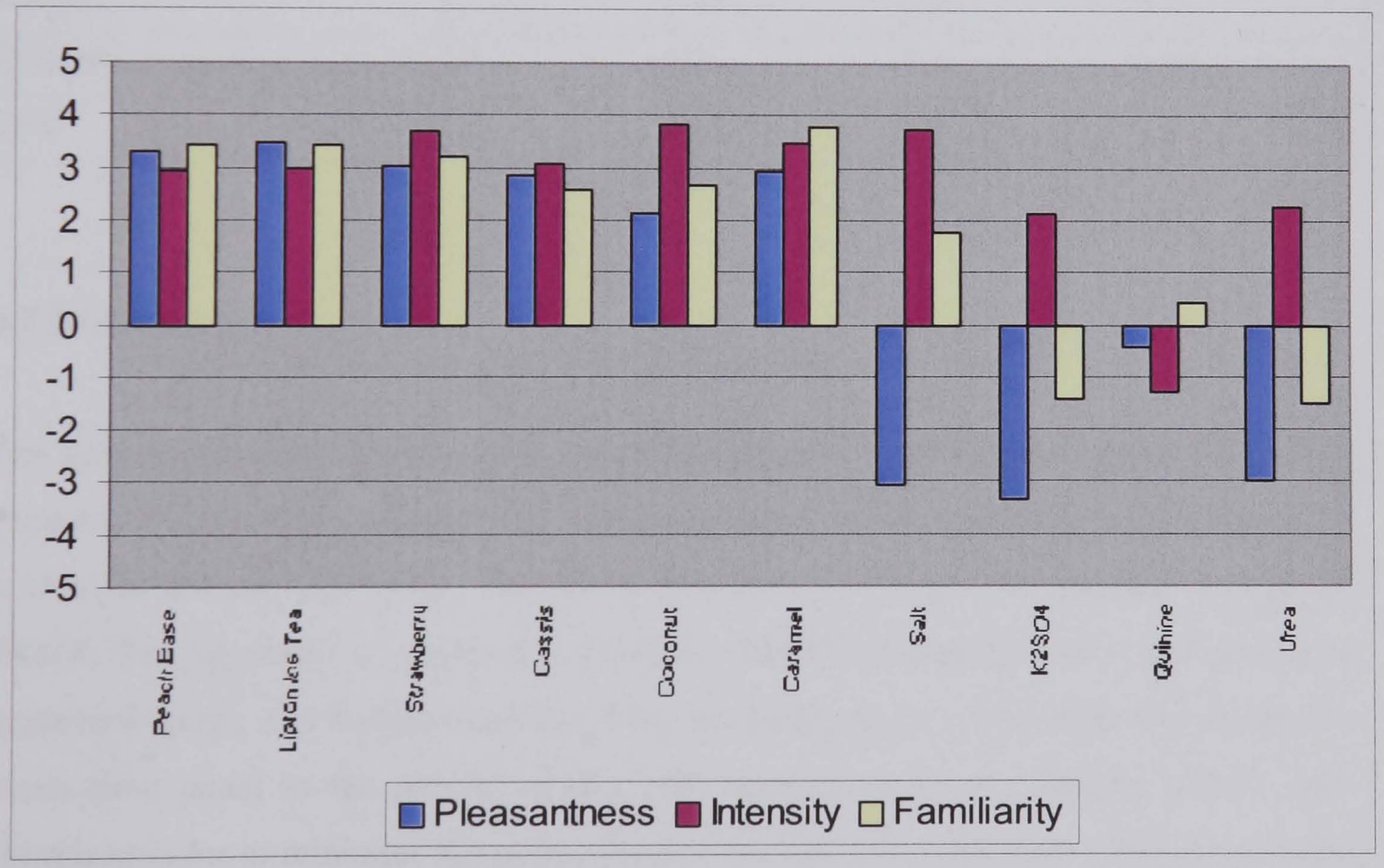
24 male subjects (mean age 28.87 years, 20 non-smokers, 4 smokers) rated the category, pleasantness, intensity and familiarity of 10 different tastes. Male subjects were chosen for the ratings to avoid the possible confounds of sex differences in the rating of tastes. All tastes were presented in cups with lids so that the subjects could not see the colour of the liquid, and also to reduce the odour component of the flavour. Subjects were asked to take a small sip and then fill in the questionnaire. Subjects were required to have some mineral water in between each taste. The order of taste presentation was randomised between subjects.

The concentrations of the stimuli were as follows: Peach base 100%, Lipton peach flavoured ice tea 100%, Strawberry 25% (Sirop de Fraise, Lejay-Lagoute, Dijon, France), Cassis 20% (Sirop de Cassis, Lejay-Lagoute, Dijon, France), Coconut 20% (Noix de Coco Sirop concentre, Lejay-Lagoute, Dijon, France), Caramel 25% (Fontana Caramel Premium Syrup, manufactured for tarbucks Coffee Company, Seattle, USA), NaCl 0.342M (Best-In table salt, packed for Bestway C&C Ltd, London, UK), K_2SO_4

0.138M (BDH Laboratory Supplies, Poole, UK), Quinine $5.4x10^{-5}$M (Quinine sulphate, BDH Laboratory Supplies, Poole, UK), Urea 0.5M (BDH Laboratory Supplies, Poole, UK). All stimuli except peach base and Lipton's peach flavoured ice tea were diluted in distilled water.

The results of the rating are shown in figure 6.2.2.1 and in table 6.2.2.1a. The criteria for stimulus selection for inclusion in the gustatory fMRI study were either very high or very low pleasantness ratings and clear category ratings. The tastes chosen for the gustatory fMRI were Lipton's peach flavoured ice tea as the pleasant taste, and two salt (NaCl and K_2SO_4) solutions as unpleasant and disgusting. Subjects were included in the fMRI study only if they perceived one of the salt solutions as disgusting and the other as unpleasant.

Figure 6.2.2.1: Pleasantness, intensity and familiarity ratings for tastes
-5 very unpleasant to 5 very pleasant, -5 very weak to 5 very intensive, -5 very unfamiliar to 5 very familiar



In the fMRI experiments artificial saliva was used as a neutral stimulus as water can be perceived as pleasant (Brunstrom et al., 1997) and has been shown to induce the same cortical activation patterns as other gustatory stimuli (de Araujo et al., 2003). Artificial

saliva has been used in previous neuroimaging studies of gustation (Francis et al., 1999; O'Doherty et al., 2001). The composition of the artificial saliva used in this study was based on this literature and contained 1.864g KCl and 0.168g $NaHCO_3$ per litre.

Table 6.2.2.1: Category ratings for tastes
Showing the category ratings for the olfactory stimuli (number of subjects who chose each category)

	Fruity pleasant	Non-fruity pleasant	Disgusting	Unpleasant non-disgusting	Neutral
Peach Base	24				
Lipton Ice Tea	24				
Strawberry	23	1			
Cassis	23	1			
Coconut	13	9	1	1	
Caramel	8	13	1	2	
Salt		1	10	13	
K_2SO_4			13	10	1
Quinine		2	3	6	13
Urea		1	12	8	2

6.2.3 Gustometer

The gustometer used to deliver the tastes was purpose-built for this experiment. The gustometer contained syringes with the liquid tastes, which were driven by computer-controlled motors (RS, UK). The motor gear box was 12V DC and had a ratio of 9RPM. The apparatus was placed in the MR control room as the motor and gear box contained metal, and Teflon tubes ran from the syringes via a filtered connector in the penetration panel to the subject in the MRI scanner (figure 6.2.3a&b). Teflon was chosen in order to minimise the permeation of the tastes into the tubes. The tastes were delivered to the subject at a rate of 5ml per minute.

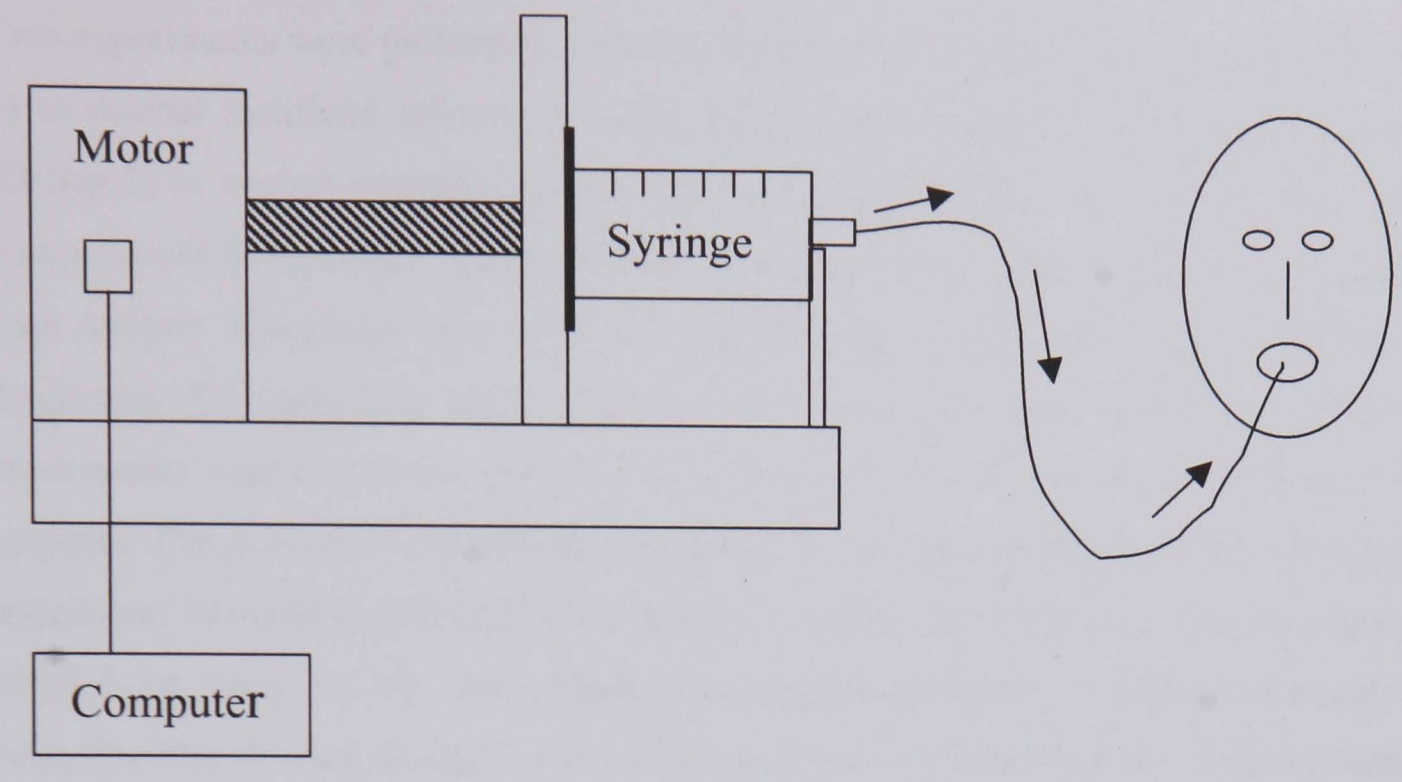


Figure 6.2.3a: Schematic illustrating the gustometer.
Not to scale. The direction of flow is indicated by arrows.

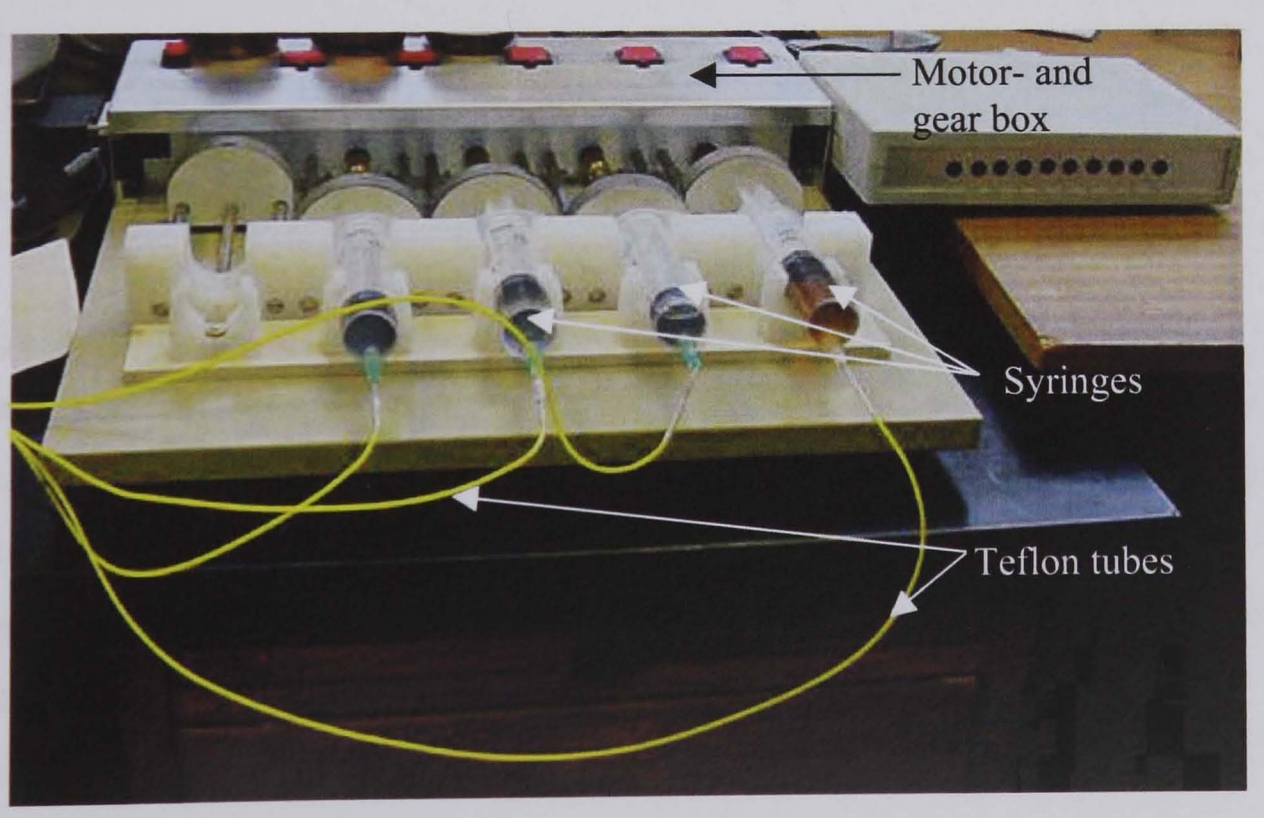


Figure 6.2.3.b: Photograph showing the gustometer.
There is space for 5 syringes containing liquid tastes. Each syringe is connected to a Teflon tube leading through a filtered connector in the penetration panel to the subject. The computer-controlled motors control which taste is delivered.

6.2.4 Experimental Design

Two experiments were performed, one comparing pleasant and disgusting tastes (Group 1) to neutral (artificial saliva), the other comparing unpleasant and disgusting tastes (Group 2) to neutral (artificial saliva). Subjects were asked to eat 2 hours prior to the scan to avoid being hungry and to refrain from caffeine prior to the scan. In the scanner each subject completed two 5-minute experiments. Each subject was exposed to disgusting (D) tastes and either pleasant (P) or unpleasant (U) tastes. The order of experiments was P/U D for half of the subjects and D P/U for the other half of the subjects. The 5-minute experiments comprised blocks of 30s ON (either D or P or U tastes) and 30s OFF (artificial saliva). During each ON phase the taste was delivered in small drops every 3s. The motor pushed the syringe forward for 300ms at the start of every 3s. The amount of liquid dispensed during those 300ms was too small to form a drop, but as the Teflon tubes were resting on the subject's tongue the liquid was expelled from the tube. Subjects were instructed not to suck on the tubes in order to avoid uneven delivery of taste stimuli, to keep swallowing to a minimum to reduce movement artefacts, and to keep their eyes closed. As taste stimuli were delivered in an identical way during both ON and OFF phases non-taste factors that could influence the results were reduced. The amount of swallowing was evenly distributed over the whole 5 min experiment.

6.2.5 Image Analysis

As subjects had to swallow the liquids during the fMRI experiment the initial analysis was performed using the updated version of the Generic Brain Activation Mapping software, as described in section 5.2.6., due to its superior ability to deal with movement artefacts. The direct comparisons of SSQ values between brain areas activated in response to different tastes (pleasant, unpleasant or disgusting) were made as described in section 5.2.6.

6.3 Results

6.3.1 Generic brain activation maps

Group 1: Major regions of generic activation in response to disgusting tastes

Generic activation was demonstrated in the right inferior temporal lobe, the hippocampus, and the left putamen in response to disgusting tastes. Areas of activation in response to the neutral condition include right dorsal frontal lobe, and left temporal and visual areas (figure 6.3.1a, table 6.3.1a).

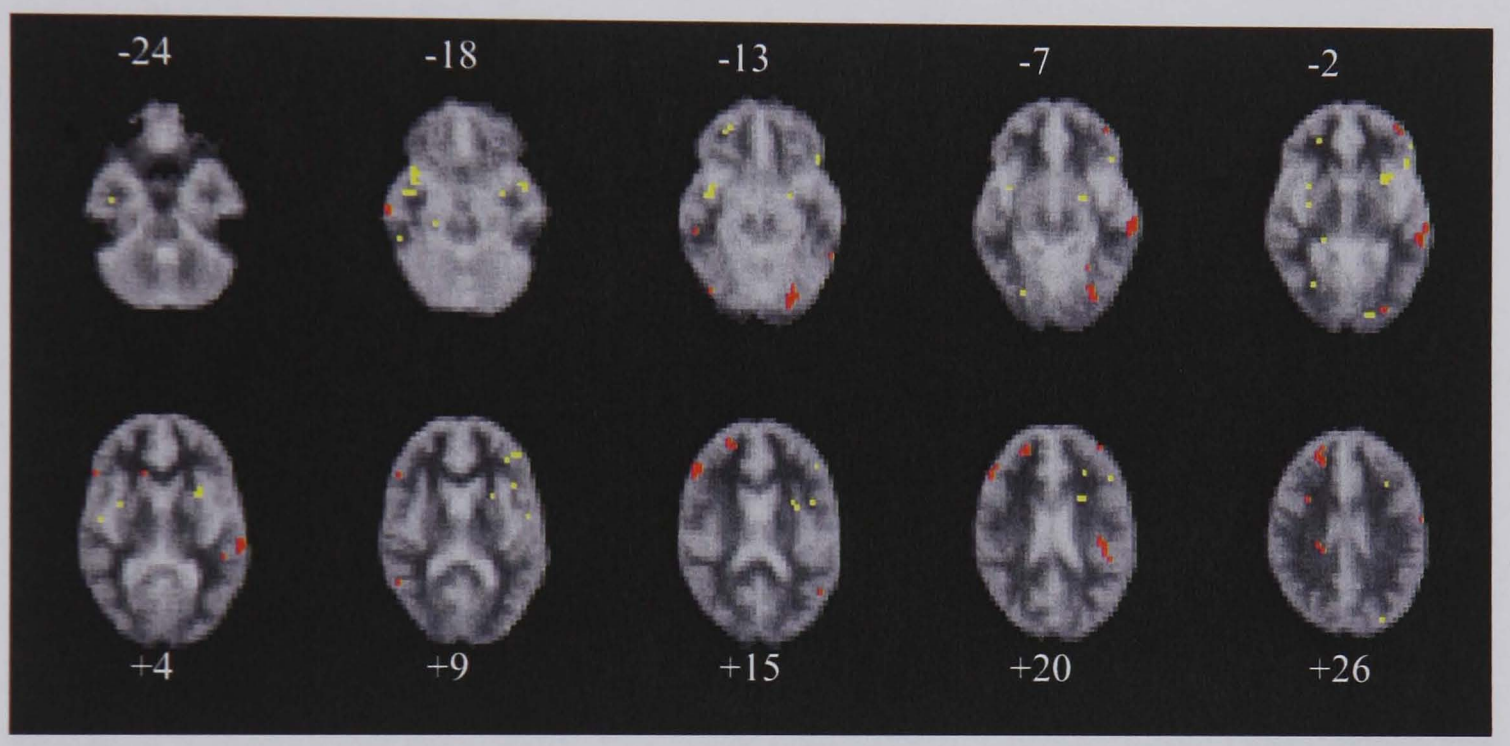


Figure 6.3.1a: Foci of generic brain activation in eight right-handed male subjects during perception of disgusting tastes in group 1.
The numbers above and below the transverse sections indicate the distance in mm from the transcallosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by disgusting tastes are shown in yellow, and voxels activated at $p<0.004$ during the neutral condition are shown in red.

Group 1: Major regions of generic activation in response to pleasant tastes

Main regions of generic brain activation in response to pleasant tastes include frontal and prefrontal cortices, such as dorsolateral prefrontal cortex (BA 46), dorsomedial prefrontal cortex (BA32) and inferior frontal gyrus (BA 45). Insular cortex and parahippocampal gyrus activation was also observed in response to pleasant tastes. The response to neutral taste was dominated by activation in posterior cingulate gyrus and visual association areas (figure 6.3.1b and table 6.3.1b).

Table 6.3.1a: Main generically activated brain regions in response to disgusting tastes in group 1.

Cerebral Region (Brodman Area)	Side	x[a]	y[a]	z[a]	No. of voxels	Condition of signal increase[b]
Inferior Temporal Lobe (BA 21)	R	40	0	-18	6	Disgust
Hippocampus	R	40	-10	-13	5	Disgust
Putamen	L	-28	7	-2	5	Disgust
Cerebellum	L	-25	-69	-13	8	Neutral
Middle Temporal Gyrus (BA 21)	L	-61	-26	-2	7	Neutral
Inferior Parietal Lobule (BA 40)	L	-36	-23	20	6	Neutral
Medial Frontal Lobe (BA 32)	R	17	37	31	6	Neutral
Inferior Frontal Gyrus (BA 45)	R	47	23	15	5	Neutral
Primary Visual Cortex (BA 18)	L	-28	-69	-7	5	Neutral
Superior Temporal Gyrus (BA 22)	L	-61	-26	4	5	Neutral

[a] The cluster with the largest number of voxels in each region is reported. Talairach coordinates refer to the voxel with the maximum Sum of Square Ratio (SSQratio) in each regional cluster. All activated voxels were identified by a one-tailed test against the null hypothesis that median SSQratio is not determined by experimental design. The probability threshold for activation was $p \leq 0.004$, this means the expected type 1 error rate is 4 false positive voxels per slice.

[b] Signal increase was detected either during presentation of disgusting/ unpleasant/pleasant tastes or neutral baseline (artificial saliva) taste.

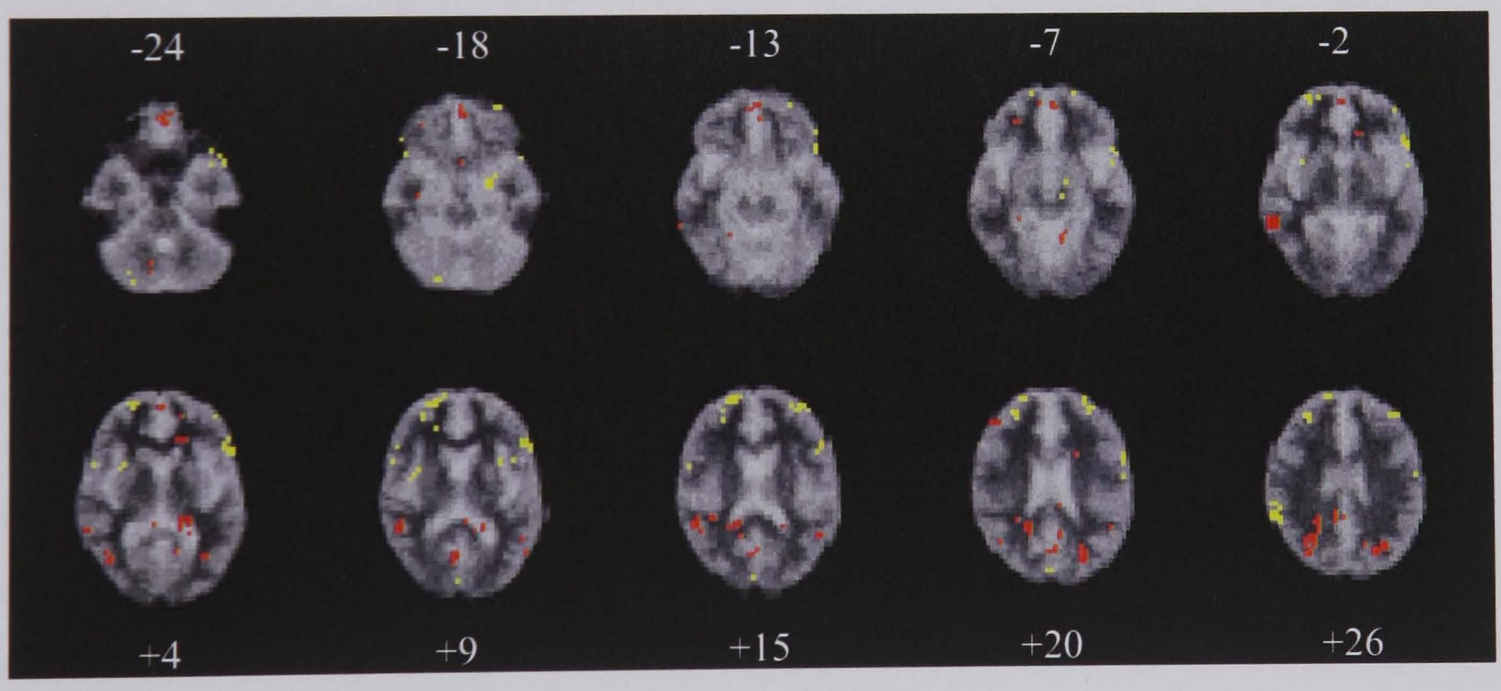


Figure 6.3.1b: Foci of generic brain activation in eight right-handed male subjects during perception of pleasant tastes in group 1.
The numbers above and below the transverse sections indicate the distance in mm from the transcallosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by pleasant tastes are shown in yellow, and voxels activated at $p<0.004$ during the neutral condition are shown in red.

Table 6.3.1b: Main generically activated brain regions in response to pleasant tastes in group 1.

Cerebral Region (Brodman Area)	Side	x[a]	y[a]	z[a]	No. of voxels	Condition of signal increase[b]
Supramarginal Gyrus (BA 40)	L/R	-50	-46	37	8	Pleasant
		53	-46	26	6	Pleasant
Inferior Frontal Gyrus (BA 45)	L	-50	13	4	7	Pleasant
Medial Frontal Lobe (BA 32)	L/R	21	43	15	7	Pleasant
		-28	37	20	5	Pleasant
Dorsolateral Prefrontal Cortex (BA 46)	L	-32	39	15	5	Pleasant
Insula	L	-40	10	9	3	Pleasant
Parahippocampal Gyrus (BA 28)	L	-21	-13	-18	3	Pleasant
Post Cingulate Gyrus (BA 23, 31, 30)	L/R	21	-43	26	15	Neutral
		-21	-63	20	7	Neutral
		4	-63	9	5	Neutral
		15	-43	15	5	Neutral
Hippocampus	L	-21	-37	4	6	Neutral
Orbitofrontal Cortex (BA 11)	L	-7	39	-24	6	Neutral
Superior Temporal Gyrus (BA 22)	R	47	-39	15	6	Neutral
Middle Temporal Gyrus (BA 21)	R	53	-39	-2	6	Neutral

[a] The cluster with the largest number of voxels in each region is reported. Talairach coordinates refer to the voxel with the maximum Sum of Square Ratio (SSQratio) in each regional cluster. All activated voxels were identified by a one-tailed test against the null hypothesis that median SSQratio is not determined by experimental design. The probability threshold for activation was $p \leq 0.004$, this means the expected type 1 error rate is 4 false positive voxels per slice.

[b] Signal increase was detected either during presentation of disgusting/ unpleasant/pleasant tastes or neutral baseline (artificial saliva) taste.

Group 2: Major regions of generic activation in response to disgusting tastes

Generic activation was demonstrated in the primary visual cortex (BA 18) and in the left mid-insula in response to disgusting gustatory stimuli. Only the cerebellum was activated significantly in response to the neutral condition (figure 6.3.1c, table 6.3.1c).

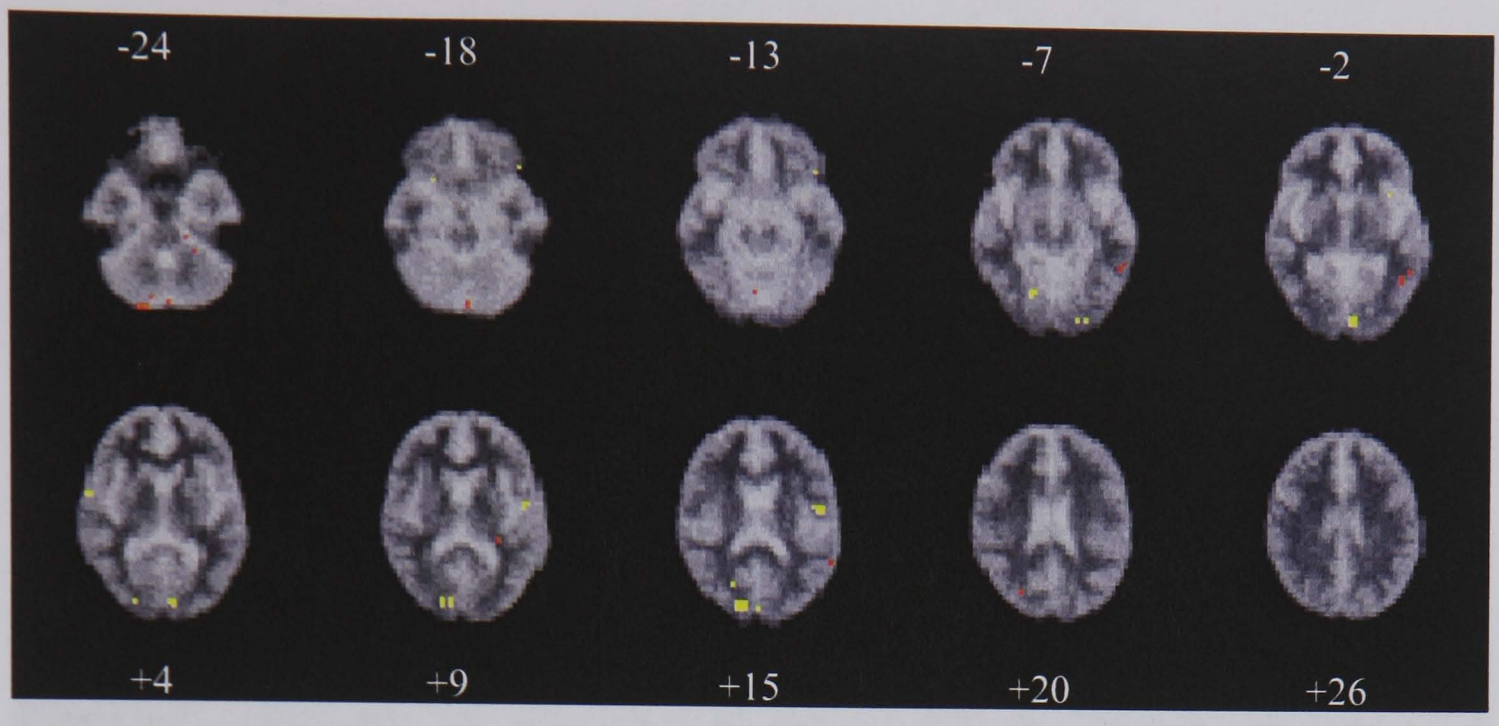


Figure 6.3.1c: Foci of generic brain activation in eight right-handed male subjects during perception of disgusting tastes in group 2.
The numbers above and below the transverse sections indicate the distance in mm from the transcallosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by disgusting tastes are shown in yellow, and voxels activated at $p<0.004$ during the neutral condition are shown in red.

Table 6.3.1c: Main generically activated brain regions in response to disgusting tastes in group 2.

Cerebral Region (Brodman Area)	Side	x[a]	y[a]	z[a]	No. of voxels	Condition of signal increase[b]
Primary Visual Cortex (BA 18)	L/R	11	-82	15	6	Disgust
		-4	-86	-2	4	Disgust
Insula	L	-47	-13	9	3	Disgust
Cerebellum	L/R	-32	-60	-40	10	Neutral
		21	-82	-29	5	Neutral

[a] The cluster with the largest number of voxels in each region is reported. Talairach coordinates refer to the voxel with the maximum Sum of Square Ratio (SSQratio) in each regional cluster. All activated voxels were identified by a one-tailed test against the null hypothesis that median SSQratio is not determined by experimental design. The probability threshold for activation was $p\leq0.004$, this means the expected type 1 error rate is 4 false positive voxels per slice.
[b] Signal increase was detected either during presentation of disgusting/ unpleasant/pleasant tastes or neutral baseline (artificial saliva) taste.

Group 2: Major regions of generic activation in response to unpleasant tastes

There were no areas of significant brain activation in response to unpleasant taste stimuli. Main regions of generic brain activation in response to neutral tastes were the medial frontal lobe (BA 32), left anterior cingulate gyrus (BA 24), left dorsolateral prefrontal cortex (BA 9) and the left thalamus (figure 6.3.1d and table 6.3.1d).

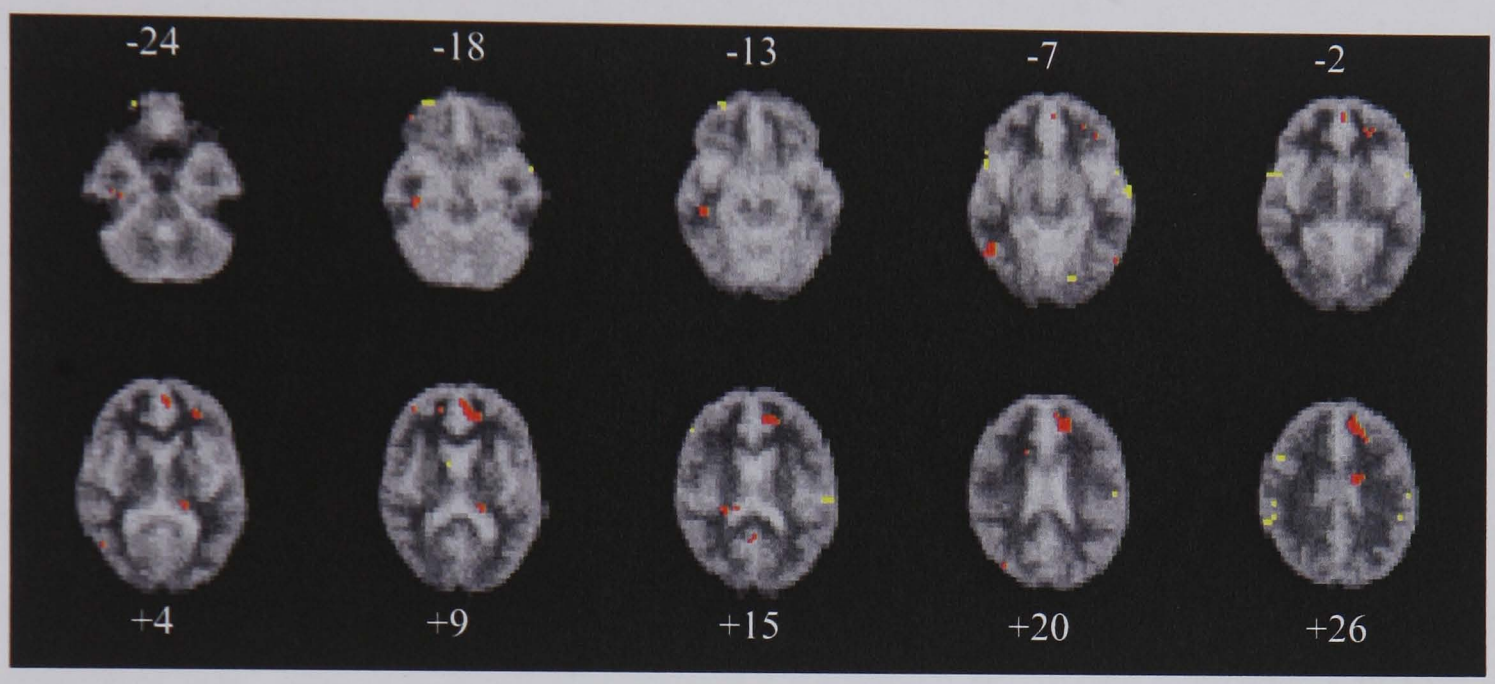


Figure 6.3.1d: Foci of generic brain activation in eight right-handed male subjects during perception of unpleasant tastes in group 2.
The numbers above and below the transverse sections indicate the distance in mm from the transcallosal line. The right side of the brain is shown on the left side of each panel, and vice versa. Voxels activated at $p<0.004$ by unpleasant tastes are shown in yellow, and voxels activated at $p<0.004$ during the neutral condition are shown in red.

Table 6.3.1d: Main generically activated brain regions in response to unpleasant tastes in group 2.

Cerebral Region (Brodman Area)	Side	x[a]	y[a]	z[a]	No. of voxels	Condition of signal increase[b]
Medial Frontal Lobe (BA 32)	L/R	0	43	9	12	Neutral
		-11	37	26	11	Neutral
Anterior Cingulate Gyrus (BA 24)	L	-17	-7	26	5	Neutral
Dorsolateral Prefrontal Cortex (BA 9)	L	-32	17	31	5	Neutral
Thalamus	L	-17	-33	4	3	Neutral

[a] The cluster with the largest number of voxels in each region is reported. Talairach coordinates refer to the voxel with the maximum Sum of Square Ratio (SSQratio) in each regional cluster. All activated voxels were identified by a one-tailed test against the null hypothesis that median SSQratio is not determined by experimental design. The probability threshold for activation was $p\leq0.004$, this means the expected type 1 error rate is 4 false positive voxels per slice.

[b] Signal increase was detected either during presentation of disgusting/ unpleasant/pleasant tastes or neutral baseline (artificial saliva) taste.

6.3.2 Within-group comparison of activation in anterior insula and putamen

Both in Group 1 and in Group 2, the comparison of a measure of the mean intensity of activation within the anterior insula and ventral putamen across conditions did not reveal a significant difference in response to disgusting compared with pleasant tastes (Group 1) and disgusting compared to unpleasant tastes (Group 2) at $p = 0.05$. The Talairach coordinates for the centres of the clusters that were included in this analysis are: a) left ventral putamen $x = -28$, $y = 13$, $z = -2$; b) left putamen $x = -27$, $y = 12$, $z = 4$; c) right dorsal middle insula $x = 36$, $y = 0$, $z = 9$; d) left dorsal posterior insula $x = -47$, $y = -5$, $z = 9$.

6.4 Discussion

The first aim of this experiment was to design a gustometer that can be used in an fMRI environment, and that is capable of delivering several different taste stimuli at a controlled rate. This was achieved by combining motor-driven syringes with software controlling the motors. The software was written in visual basic and allowed control of the time that a motor would push an individual syringe forward. By measuring the amount of liquid over a minute of continuous motor activity it was possible to establish flow rates. With this gustometer it is also possible to adjust the flow rate by altering the time a syringe is pushed forward by a motor and the time in between motor activity. It is also possible to run the paradigm as performed in the scanner and measure the amount of liquid delivered throughout an experiment. This set-up allows quite precise control over the amounts of liquid delivered and the timing of the stimulus delivery. A major concern of delivering liquid stimuli to subjects in the fMRI scanner was a possible choking hazard. It was found that only small amounts of liquid were necessary (5ml per minute) to produce a taste sensation, greatly reducing the possibility of a subject feeling discomfort or choking. Delivering small amounts of liquid also reduces swallowing, which can increase movement artefacts. In addition, delivery of small amounts can reduce the odorous component of flavours such as the peach-flavoured ice tea used as a pleasant stimulus.

The other aim of this experiment was to investigate the extent to which the insula cortex is differentially activated in response to pleasant, unpleasant and disgusting tastes in

order to establish if the anterior insula is involved in the perception of disgust across sensory modalities. Based on lesion and electrophysiological studies, the frontal operculum and part of the dorsal anterior insula are thought to be the primary gustatory cortex in both non-human and human primates (Ogawa, 1994). This has been supported by functional neuroimaging studies in humans (O'Doherty et al., 2001; Small et al., 1999) and in non-human primates (Kobayashi et al., 2002). One fMRI study has reported ventral rather than dorsal anterior insula activation in response to tastes (King et al., 1999). The hypothesis for the experiment described in this chapter was that there would be insula activation in response to all taste stimuli, with significantly increased activation in response to disgusting tastes compared to pleasant or unpleasant tastes.

The results of these experiments did not consistently support this hypothesis. There was no consistent activation of the insula in response to tastes, with some insula activation in response to pleasant tastes (table 6.3.1b) and in response to disgusting tastes in group 2 (table 6.3.1c), but not in response to disgusting tastes in group 1 (table 6.3.1a) or in response to unpleasant tastes (table 6.3.1d). The insula activation in response to disgusting tastes in group 2 is in the middle to posterior insula rather than in the anterior insula as predicted. There is activation in the ventral putamen in response to disgusting tastes in group 1, which is in a similar location to the ventral striatal activation in response to disgusting odours observed in chapter 5.

Most neuroimaging studies of gustation report more robust insula activation in response to tastes *per se* than observed here (Faurion et al., 1999; Frey & Petrides, 1999; O'Doherty et al., 2001; Small et al., 1999; Zald et al., 1998, 2002). However, the insula activation that was observed in response to pleasant tastes was in the dorsal insula close to the frontal operculum, which is in agreement with the literature (Faurion et al., 1999; Francis et al., 1999; Frey & Petrides, 1999; Ogawa, 1994; Zald et al., 1998). Small et al. (1997b) also failed to find insula activation in response to a gustatory stimulus (citric acid); the authors suggested this might be due to identical somatosensory stimulation of the mouth in both the citric acid condition and the control condition (water). It is also possible that their lack of insula activation was due not to identical somatosensory stimulation in response to both citric acid and water, but because water has been shown to activate similar human cortical gustatory areas as other taste stimuli (de Araujo et al., 2003). This is why in this study as in other previous neuroimaging studies of taste (de

Araujo et al., 2003; Francis et al., 1999; O'Doherty et al., 2001) artificial saliva was used as a control stimulus instead of water.

Activation was also expected in the secondary gustatory cortex, the orbitofrontal cortex (O'Doherty et al., 2001; Ogawa, 1994; Rolls & Baylis, 1994; Small et al., 1999). However, no activation was observed in the orbitofrontal cortex in response to tastes. This could be due to the acquisition parameters used in this study, which do not favour the orbitofrontal cortex. Another fMRI study (Faurion et al., 1999) using 6mm thick axial slices also failed to show activation in the secondary gustatory cortex, although they used a 3T MRI scanner which causes more pronounced signal drop out than a 1.5T MRI scanner. 7mm thick axial slices as used in this experiment can easily lead to signal drop-out in the orbitofrontal cortex (see figure 5.4). Most previous taste studies have used PET (Small et al., 1999), but some recent studies using fMRI have tried to optimise orbitofrontal cortex acquisition by using coronal slices (de Araujo et al., 2003; Francis et al., 1999; O'Doherty et al., 2001), minimizing voxel size, high gradient switching frequency, a short echo time, and individual shimming (de Araujo et al., 2003; O'Doherty et al., 2001). As the acquisition parameters were kept identical across experiments in this thesis to allow comparison of results only some of these optimisations were possible. The in-plane resolution is kept relatively small here, too, at 3.8mm. EPI suffers from some geometric distortions, the magnitude of which increases with increasing field strength. Therefore the gradient switching frequency did not need to be as high here, as the experiments were performed using a 1.5T scanner unlike the other studies (de Araujo et al., 2003; O'Doherty et al., 2001), which used a 3T scanner. Each subject is individually shimmed at the Maudsley where my experiments were performed. Nevertheless, this does not seem enough to address the signal drop-out observed in the orbitofrontal cortex. In addition, the main area of interest was the insula cortex, which is covered by the acquisition parameters used in this study.

One possibility is that there was little activation overall because the stimuli were delivered in small quantities and therefore were more like pure tastes than flavours, which are a combination of tastes and odours. The unpleasant and disgusting stimuli had no olfactory component to them as they were salt solutions. It is, though, possible that the stimuli used were not sufficiently emotionally salient due to the lack of an olfactory component to the stimuli. Furthermore, the only stimulus which might have had an

olfactory component, and therefore could have been a flavour instead of a taste, the pleasant peach-flavoured ice tea, led to the greatest activation both within the insula and overall. Another possibility is that the observed difference in activation could be due to differences in the familiarity of the tastes used (figure 6.2.2.1), as subjects rated peach-flavoured ice tea and salt solution as familiar, but not K_2SO_4. It is also worth noting that during the initial rating of tastes there was no clear distinction between disgusting and unpleasant non-disgusting tastes (table 6.2.2.1a). It is possible that although subjects labelled a taste as disgusting, this stimulus was not powerful enough to actually lead to increased blood flow to the anterior insula, especially the right anterior insula, maybe due to a lack of experiencing disgust. Another confound worth taking into account in future experiments is the possible difference between subjects in their PROP (6-n-propylthiouracil) status and associated with this, whether subjects are sucrose likers or dislikers. This has been shown to have an influence on how pleasant or unpleasant tastes are perceived (Looy et al., 1992), and might therefore have an effect on the neural regions activated in response to certain tastes.

The data presented in this chapter differ considerably from the results obtained with olfactory stimuli (chapter 5). Left anterior insula activation was observed in response to all olfactory stimuli. Right anterior insula and right ventral putamen were activated by disgusting odours only. In response to tastes, however, the pattern of activation was different. In response to disgusting tastes, activation was observed in the left middle insula in group 2, and in the left ventral putamen in group 1. This is closer to the pattern of insula and ventral putamen activation observed with facial expressions of disgust (Phillips et al., 1998b, 1999; also see chapter 7). Part of the putamen borders the medial insula, and the activation could extend to the medial surface of the insula.

Activation was also observed in the left putamen in response to disgusting odours. However, the area, which was activated to a significantly greater extent in response to disgusting odours as compared to unpleasant odours was in the right putamen and not in the left putamen. There was no insula or ventral putamen activation in response to unpleasant gustatory stimuli. These results might suggest that the tastes used in this experiment were not perceived as disgusting, and that tastes need an olfactory component to turn them into flavours which can be perceived as disgusting. However, this seems unlikely, as a study (Small et al., 1997a) investigating the differences in

activation between odours, tastes and flavours found less activation in olfactory and gustatory areas of the brain in response to flavours than in response to odours or tastes alone. In non-human primates it has been shown that there are neurons in the orbitofrontal taste area, which also respond to stimuli from other sensory modalities, for example vision and olfaction (Rolls, 1999). It has therefore been suggested that it is in these orbitofrontal cortex areas where a representation of flavour is built. No orbitofrontal activation was observed in this study, which could be due to the data acquisition as discussed above, but could also indicate that there was no flavour representation, and therefore that the tastes used were pure tastes. The findings from the study presented in this chapter are contradictory to the results by Small et al. (1997a), and further investigation is needed to determine the extent to which activation in response to odours, tastes and flavours overlaps.

Overall, it is difficult to reconcile the findings from this chapter with the literature. Three previous studies investigated the neural response to pleasant and unpleasant tastes (O'Doherty et al., 2001; Zald et al., 1998, 2002). Two report activation of the insula, orbitofrontal cortex and amygdala in response to both pleasant and unpleasant tastes (O'Doherty et al., 2001; Zald et al., 2002) when contrasted with a neutral stimulus, and one reports orbitofrontal cortex and amygdala activation in response to aversive tastes as compared to water, and insula activation in response to an aversive taste compared to chocolate (Zald et al., 1998). Interestingly, the pleasant taste contrasted with neutral did not lead to an increase in rCBF in the insula, orbitofrontal cortex or amygdala in this study (Zald et al., 1998). These results could be due to water being used as the neutral stimulus, as water does activate brain areas similar to other tastes (de Araujo et al., 2003), and can also act as a positive reinforcer (Zald et al., 1998). Another study (Small et al., 2001) investigated brain activity in response to eating chocolate beyond satiety and therefore changing its reward value from pleasant to unpleasant. When the subjects rated the chocolate as pleasant, activation was found in the caudomedial orbitofrontal cortex and the insula/frontal operculum, whereas when subjects ate chocolate despite being satiated activation was observed in the caudolateral orbitofrontal cortex but not in the insula/frontal operculum. Based on these previous studies investigating neural correlates of pleasant and unpleasant taste, it appears that both pleasant and unpleasant tastes activate the anterior insula/frontal operculum, the orbitofrontal cortex and the amygdala. It has been suggested that pleasant and unpleasant tastes may be processed in

different areas of the orbitofrontal cortex (O'Doherty et al., 2001; Small et al., 2001), with pleasant tastes causing increased blood flow to the caudomedial orbitofrontal cortex and unpleasant tastes to the caudolateral orbitofrontal cortex. Unfortunately, these results could not be replicated in this study.

A future study investigating the neural correlates underlying the perception of taste, smell and flavour could use a set of olfactory and gustatory stimuli, which can be combined to yield a flavour. If only one flavour were to be investigated this would ensure the same familiarity and intensity ratings for all stimuli. Stimulus delivery should preferably be performed in an identical way for all three (gustatory, olfactory and flavour) stimuli, which could be achieved by orally delivering all stimuli, even the odours by relying on retronasal pathways. Data acquisition should ensure coverage of the temporal and orbitofrontal lobes. In this way it would be possible to investigate the different brain areas involved in the perception of tastes and odours, and to explore if these two modalities also combine in the human orbitofrontal cortex to yield a representation of flavour.

In summary, it is difficult to conclude from the findings presented in this chapter whether there is anterior insula activation in response to disgusting tastes, which is different from or greater than insula activation observed in response to tastes per se. The results could also suggest that there is little or no disgust perception with pure taste stimuli that lack an olfactory component, as no pronounced anterior insula activation was observed in response to disgusting tastes. No previous study has investigated the neural correlates of disgusting tastes. The neural basis underlying different tastes, especially disgusting ones, and the relationship of brain activation in response to tastes with activation in response to flavours, requires further investigation.

Chapter 7

Integration of Results from Experimental Chapters

7.1 Introduction

It is important in evolutionary terms that an appropriate behavioural response be made to an emotionally-salient stimulus regardless of the sensory modality in which the stimulus is presented. Emotional processing appears to depend on separate yet overlapping neural networks. As described in section 1.3, emotion perception seems to be dependent upon the functioning of two parallel neural systems: a ventral, or "limbic", system, which is important for the identification of emotional information and the generation of affect states; and a dorsal system, which plays a role in the performance of executive functions, including selective attention, planning, and motor responses to emotional stimuli, and includes regions where cognitive processes are integrated with and can be biased by emotional input. In this chapter I am going to focus on the neural network underlying the identification of emotional stimuli from the environment, and on the overlap of neural correlates of perception of a specific emotion, disgust, regardless of sensory modality of stimulus presentation.

Several neuroimaging studies have attempted to delineate the cortical and subcortical regions involved in processing emotionally valenced stimuli in humans. Such studies have examined responses to pleasant and/or unpleasant visual (Irwin et al., 1996; Morris et al., 1996; Lane et al., 1997a&b; Phillips et al., 1998b; Whalen et al, 1998; Taylor et al., 2000), auditory (Imaizumi et al., 1997; Blood et al., 1999; Zald & Pardo 2002), olfactory (Zald & Pardo 1997; Fulbright et al., 1998; Royet et al., 2000), gustatory (Zald et al., 1998, 2002; Small et al., 1999, 2001; O'Doherty et al., 2001), and somatosensory (Francis et al, 1999; Aziz et al., 2000) stimuli. These findings are further support for the roles of limbic, frontal and sensory cortical regions in the perception of emotionally salient stimuli, but further studies are required to increase understanding of the nature of the neural response to different types of emotional stimuli presented in different sensory modalities.

Consistent with the animal literature (LeDoux 1998, 2000), the amygdala has been observed to activate during exposure to emotionally valenced stimuli in multiple

sensory modalities, particularly during aversive stimuli. Orbitofrontal cortex activations have emerged during exposure to both pleasant and unpleasant stimuli in multiple sensory modalities. Although not quite as frequent, activations have also been localised to the hippocampal and parahippocampal region in several sensory modalities (Lane et al., 1997; Zald et al., 1998; Blood et al., 1999; Phillips et al., 1998b) (also see activations reported in sections 3.3.2, 4.5.5.2, 6.3.1). An additional brain region that has been observed in response to unpleasant, especially disgusting, stimuli in several sensory modalities is the insula (Phillips et al., 1997, 1998; Small et al., 1997; Mirz et al., 2000; Sander & Scheich 2001; Schienle et al., 2002; Zald et al., 2002; Anderson et al., 2003b).

A major difficulty in interpreting the above literature arises because of the variable methods used across studies. These studies differ in the emotional intensity and number of stimuli presented, the task performed by subjects, the subject population, and the imaging and analysis methods. It thus remains unclear to what extent variable results reflect sensory-specific engagement of different brain regions or methodological discrepancies. Even within areas that have emerged across studies using different sensory modalities, it is not known to what extent variability in the magnitude, laterality or specific location of responses, reflect sensory specific or methodological factors. One study reported differences in activation between individuals, with extraversion predicting amygdale activation in response to happy faces (Canli et al., 2002). Few studies have systematically examined the neural responses to emotional stimuli presented in two or more sensory modalities: one study investigated the neural responses to olfactory, gustatory and combined olfactory and gustatory stimuli (Small et al., 1997), one to pleasant touch, olfactory and gustatory stimuli (Francis et al., 1999), one the amygdala response to neutral faces and aversive odours (Birbaumer et al., 1998), one the neural correlates of facial and vocal emotional expressions (Phillips et al., 1998b), and one the emotional responses to visual, auditory and olfactory stimuli (Royet et al., 2000).

Small et al. (1997) used PET to investigate the neural correlates underlying olfaction, gustation, and olfaction and gustation combined. As discussed in the previous chapter, olfaction and gustation combine to produce flavour perception. In this study Small et al. reported activation of primary and secondary taste cortex in response to pure gustatory

stimuli, and activation of primary and secondary olfactory cortex and left anterior insula in response to pure olfactory stimuli. These activations seemed to be greater in response to individual olfactory or gustatory stimuli than in response to flavours. Unfortunately, results for a subtraction of a neutral baseline from flavour stimuli were not reported, and it is therefore unclear to what extent the neural networks processing olfactory and gustatory stimuli overlap. Additionally, both pleasant and unpleasant stimuli were used in this study in a mixed design and it is therefore not possible to make a distinction between brain areas involved in the perception of pleasant as compared to unpleasant olfactory and gustatory stimuli.

Another study (Birbaumer et al., 1998) reported amygdala activation in response to aversive odours in both normal controls and in social phobics, but amygdala activation in response to neutral faces only in social phobics. This study has several limitations, for example it investigated amygdala activation only. Nevertheless, amygdala activation was demonstrated in response to stimuli from both the olfactory and the visual modality.

Phillips et al. (1998b) investigated the neural responses to both visual and auditory stimuli of fear and disgust. They reported amygdala activation in response to fearful visual and auditory stimuli, but anterior insula activation only in response to visual stimuli of disgust but not to auditory stimuli of disgust. This was unexpected and is in contrast to a neuropathological study (Calder et al., 2000) in which a patient with an insula lesion was impaired at recognising both facial and vocal expressions of disgust. Furthermore, Phillips et al. reported right hippocampal activation in response to both visual and auditory fearful stimuli and proposed a role for the hippocampus in the perception of fearful stimuli, which is in accordance with other reports (Gray and McNaughton 2000; Ploghaus et al., 2001, 2003). The only region activated in response to all four emotional stimuli was the superior temporal gyrus, which has been associated with perception of faces (Kanwisher et al., 1997), and eye and mouth movements (Puce et al., 1998). These findings support a network of partly overlapping neural regions for the perception of different emotions from stimuli presented in different sensory modalities.

One study investigated neural areas involved in the perception of pleasant touch, smell and taste (Francis et al., 1999), with a focus on the orbitofrontal cortex. Compared to the

studies described above this one used three different subject groups, one for each sensory modality, whereas the others (Small et al., 1997; Birbaumer et al., 1998; Phillips et al., 1998b) have performed experiments in different sensory modalities in a single subject group. In response to pleasant and neutral touch activation was observed in the somatosensory cortex and orbitofrontal cortex, with more orbitofrontal activation in response to pleasant touch (Francis et al., 1999). Both pleasant olfactory and gustatory stimuli activated bilateral insula, anterior cingulate cortex, and orbitofrontal cortex. However, the area of the orbitofrontal cortex activated by pleasant touch differed from the areas activated by pleasant taste and by pleasant smell.

The most thorough study to date investigating emotional responses to stimuli presented in different sensory modalities is by Royet et al. (2000). In this study, neural responses to pleasant and unpleasant visual, auditory and olfactory stimuli were investigated using PET. A homogeneous subject group was used: 12 right-handed male volunteers. All stimulus presentations were synchronized with inspiration to allow for the same number of stimulus presentations to be made in each sensory modality. A weakness of this study is that both pleasant and unpleasant stimuli were presented in the same paradigm and no distinction was made between pleasant and unpleasant conditions, they were combined to yield an 'emotional' condition. During the stimulus presentations subjects had to make forced-choice pleasant and unpleasant judgments. It has been shown in previous neuroimaging studies that an overt emotional decision task can change the underlying brain activation (Critchley et al., 2000; Lange et al., 2003). Nevertheless, this study provides the first direct comparison of emotional processing in different sensory modalities. In all three sensory modalities, presentation of emotional stimuli led to an increase in regional cerebral blood flow in the left orbitofrontal cortex, the left temporal pole, and the left superior frontal gyrus (Royet et al., 2000). These results suggest that emotional judgments recruit a core network in the left hemisphere, regardless of sensory modality of stimulus presentation.

Little work has been performed investigating the neural correlates underlying the perception of a single emotion, rather than just pleasant or unpleasant, from stimuli presented in several sensory modalities (Phillips et al., 1998). This could be due to the difficulties of 'translating' emotions such as sadness or fear into sensory modalities other than vision or audition. However, the focus of this thesis is on disgust, and there is

an increasing wealth of evidence now that the insula is involved in the processing of disgusting visual stimuli (Phillips et al., 1997, 1998b, 1999, 2000; Sprengelmeyer et al., 1998; Calder et al., 2000; Schienle et al., 2002), although there is still some uncertainty concerning auditory stimuli (Phillips et al., 1998b; Calder et al., 2000, 2001). In this chapter I aim to examine the extent to which previously published areas of brain activation in response to facial expressions of disgust overlap (Phillips et al., 1997, 1998b, 1999), and to create a mask of this overlap. Furthermore, I am going to explore to what extent these areas that are involved in the processing of facial expressions of disgust overlap with brain areas involved in the processing of disgusting stimuli presented in other sensory modalities.

7.2 Methods

Three sets of previously published data (Phillips et al., 1997, 1998b, 1999) were used to establish brain areas commonly activated in response to facial expressions of disgust. All three data sets were originally obtained using the same experimental design as described in sections 3.2.2 and 3.2.3. Only data from healthy control subjects was included in this analysis.

As some data were acquired on a previous scanner (Phillips et al., 1997), this data set first had to be brought into the same format as data acquired on the current MRI scanner. The previous scanner used a matrix size of 128x64 voxels, whereas the current one has a matrix size of 64x64 voxels. To make the different data acquired on the old and the new scanner comparable, data acquired on the old scanner was resized. In the 128x64 matrix brain matter was only contained in the central 64 voxels and 32 voxels on either side were empty. Those 32 voxels were sliced off both the left and right sides of the images. Subsequently the images were rescaled linearly to standardize image sizes. With all the data sets in the same format, the activation maps were superimposed. Only areas where activation from at least two of the three data sets overlapped were included in the mask.

The overlap mask that was created as described above was then applied to the data sets acquired in the course of this thesis. Only the mask and areas of overlap between the mask and the individual experiments were included in the results.

7.3 Results

7.3.1 Creation of an overlap mask using published data

There was considerable overlap in the brain areas activated in response to facial expressions of disgust published in three different papers (Phillips et al., 1997, 1998b, 1999). No brain area was reported in all three studies, but several regions were reported in two studies (figure 7.3.1 and table 7.3.1). These areas of activation centre on the left insula and the right putamen, but also include the right superior temporal gyrus and left inferior posterior temporal gyrus (BA 37).

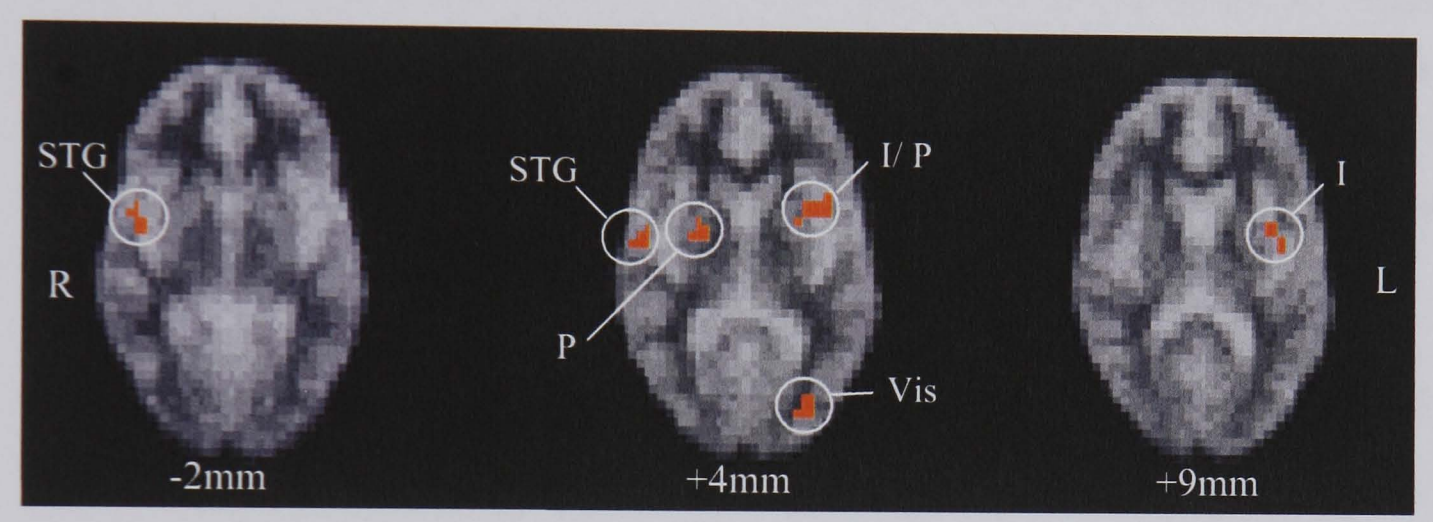


Figure 7.3.1: Overlap mask.
Overlap of brain areas activated in response to facial expressions of disgust in two out of three studies (Phillips et al., 1997, 1998b, 1999). There was no area of overlap between all three studies. The numbers below the transverse sections indicate the distance in mm from the transcallosal line. R = right hemisphere, L = left hemisphere. STG = Superior temporal gyrus, P = Putamen, I = insula, Vis = Primary visual cortex.

Table 7.3.1 Overlap mask: Areas of common activation

Region activated (approximate Brodmann area)	Side	x[a]	y[a]	z[a]	Number of activated voxels
Insula/Putamen	L	-25	4	4	10
centre of cluster[b]: Insula		-36	11	4	
Primary visual cortex (BA 18)	L	-25	-81	4	7
Superior temporal gyrus (BA 22)	R	47	-4	-2	7
Putamen	R	29	-4	4	6
Insula	L	-40	-7	9	6
Superior temporal gyrus (BA 22)	R	58	-7	4	6

[a] Talairach coordinates refer to the voxel with the maximum FPQ value in each regional cluster.
[b] Talairach coordinates for the centre of this cluster as opposed to the voxel with the maximum FPQ value, as in this case the voxel with the maximum FPQ value does not seem representative of the whole cluster.

7.3.2 Application of overlap mask to data from experimental chapters

There was some overlap between the mask created from previously published data (Phillips et al., 1997, 1998b, 1999) and the brain activation in response to disgusting stimuli presented in multiple sensory modalities (vision, audition, olfaction and gustation) for most datasets presented in this thesis. However, in some instances there was no overlap. This was the case for the data on facial expressions of disgust presented in section 3.3.2, figure 3.3.2 IIa and table 3.3.2 IIa, despite the overlap mask also having been created from data obtained by presenting facial expressions of disgust. The reasons for the lack of insula or putamen activation in response to facial expressions of disgust in the experiment described in this thesis have been discussed in section 3.4. There was also no overlap of areas of brain activation between the overlap mask and brain activation in response to disgusting tastes (section 6.3.1, group 2). Some explanations for this have been discussed in section 6.4.

Considerable overlap was observed between brain areas activated in response to non-verbal vocal expressions of disgust and the overlap mask created from brain activation in response to facial expressions of disgust (figure 7.3.2a). There was substantial overlap in the bilateral mid-anterior insula and the right superior temporal gyrus, and some overlap in the inferior posterior temporal gyrus.

Figure 7.3.2a: Overlap between mask and activation in response to auditory stimuli of disgust.
Common brain areas of activation in the mask generated from Phillips et al. data and in response to non-verbal vocal stimuli of disgust (chapter 3.3.2, table 3.3.2.IIb). The right side of the brain is depicted on the left side of the figure and vice versa. The numbers below the transverse sections indicate the distance in mm from the transcallosal line. Areas of the mask that do not overlap with activation in response to auditory stimuli of disgust are shown in orange, and the overlap is shown in yellow.

A small amount of overlap in areas of brain activation in the mask and in response to facial expressions of disgust was observed for the data presented in section 4.5.5.2. For this comparison data from the second experiment were chosen, as this was the only one of the four experiments presenting facial expressions of disgust showing reliable insula activation (figure 4.5.5.2, disgust 2). The overlap was confined to one voxel in the inferior posterior temporal gyrus.

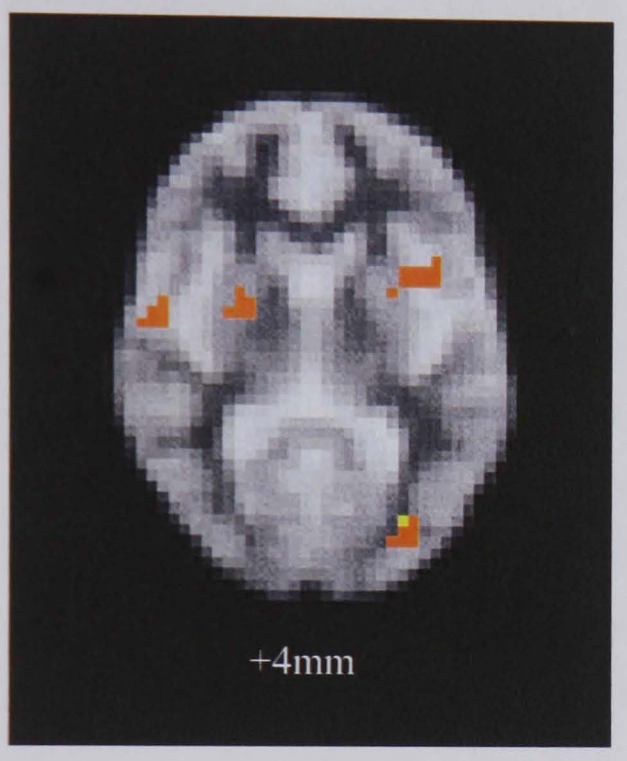


Figure 7.3.2b: Overlap between mask and activation in response to visual stimuli of disgust.
Overlap between areas of activation in the mask generated from Phillips et al. data and in response to facial expressions of disgust (section 4.5.5.2, Disgust 2). The right side of the brain is depicted on the left side of the figure and vice versa. The number below the transverse section indicates the distance in mm from the transcallosal line. The original mask is shown in orange, and the overlap is shown in yellow.

Despite similar results for both groups of subjects in chapter 5 in response to disgusting olfactory stimuli, there were discrepancies when these results were combined with the overlap mask created from published data. For group 1, who were presented with pleasant and disgusting odours, the overlap between the mask and the brain activation in response to disgusting odours was confined to one voxel in the right superior temporal gyrus (figure 7.3.2c). For group 2, who were presented with unpleasant and disgusting odours, there was much greater overlap, not in the superior temporal gyrus, but in the left insula, the right putamen, and the left inferior posterior temporal gyrus (figure 7.3.2d).

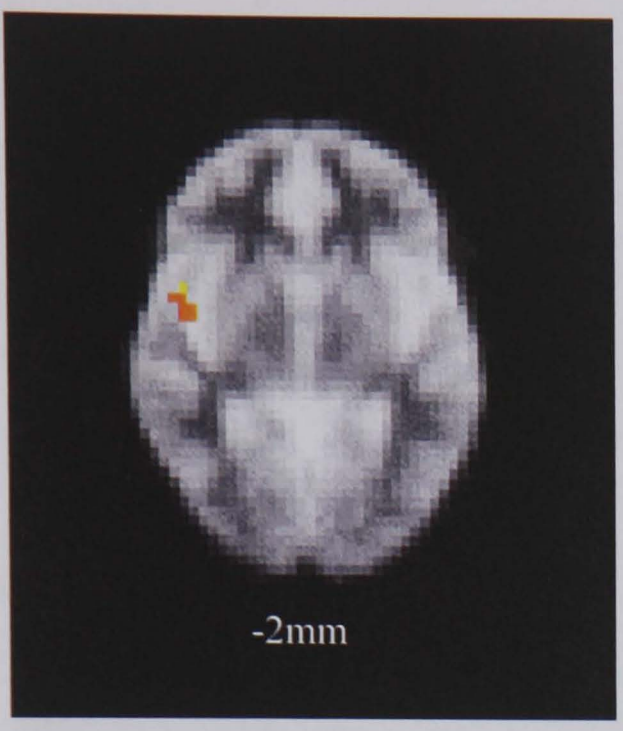


Figure 7.3.2c: Overlap between mask and activation in response to olfactory stimuli of disgust (Group 1).
Overlap between areas of activation in the mask generated from Phillips et al. data and in response to disgusting olfactory stimuli (section 5.3.1, Group 1). The right side of the brain is depicted on the left side of the figure and vice versa. The number below the transverse section indicates the distance in mm from the transcallosal line. The original mask in shown in orange, and the overlap is shown in yellow.

The brain areas activated in response to disgusting gustatory stimuli overlapped with the mask created from published activation in response to facial expressions of disgust only in group 1, who were exposed to pleasant and disgusting tastes. There was no overlap between experimental data and the mask in Group 2, who were presented with unpleasant and disgusting tastes. In Group 1, the overlap between the mask and the data from chapter 6 was confined to one voxel in the left putamen (figure 7.3.2e).

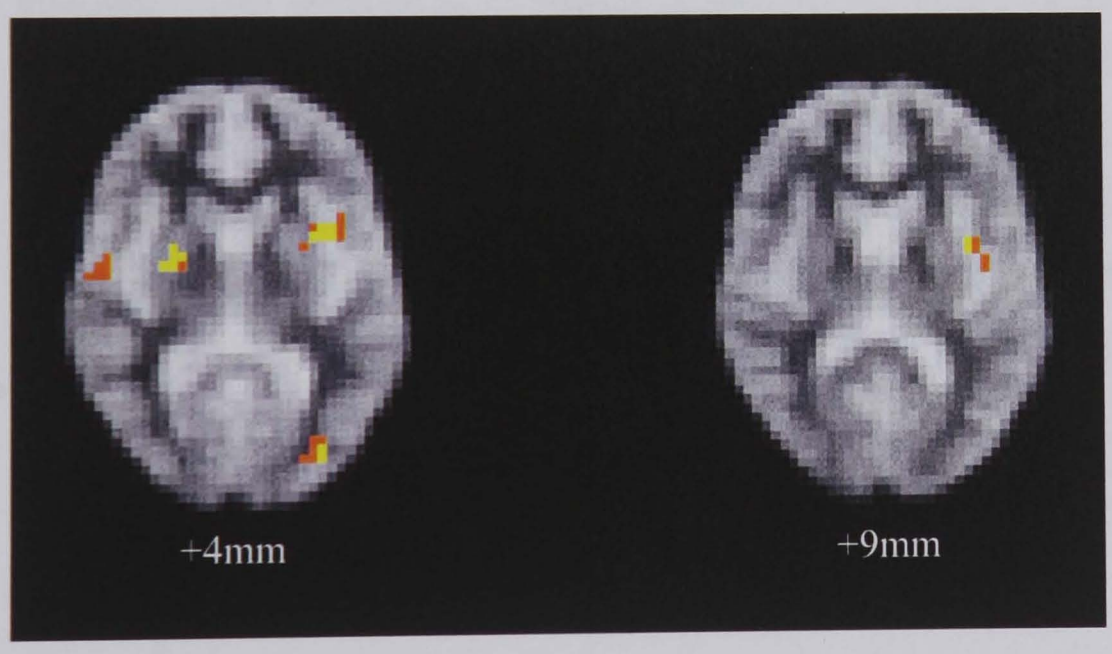


Figure 7.3.2d: Overlap between mask and activation in response to olfactory stimuli of disgust (Group 2).
Overlap between areas of activation in the mask generated from Phillips et al. data and in response to disgusting olfactory stimuli (section 5.3.1, Group 2). The right side of the brain is depicted on the left side of the figure and vice versa. The numbers below the transverse sections indicate the distance in mm from the transcallosal line. Areas of the mask that do not overlap with activation in response to auditory stimuli of disgust are in shown in orange, and the overlap is shown in yellow.

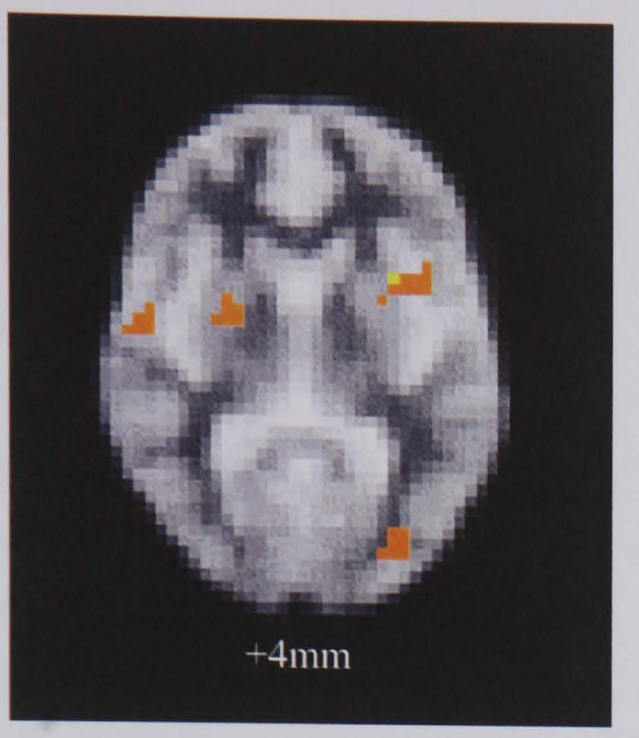


Figure 7.3.2e: Overlap between mask and activation in response to gustatory stimuli of disgust (Group 1).
Overlap between areas of activation in the mask generated from Phillips et al. data and in response to disgusting gustatory stimuli (section 6.3.1, Group 1). The right side of the brain is depicted on the left side of the figure and vice versa. The number below the transverse section indicates the distance in mm from the transcallosal line. The original mask in shown in orange, and the overlap is shown in yellow.

Summary

A mask showing areas of overlap between brain activation demonstrated in response to facial expressions of disgust was successfully created using previously published data (Phillips et al., 1997, 1998b, 1999). The areas of activation demonstrated in two out of those three data sets include the bilateral insula and the putamen, believed to be involved in the perception of disgust (Calder et al., 2001). When this mask, created entirely from brain responses to disgusting visual stimuli, was applied to data collected in response to disgusting stimuli presented in multiple sensory modalities (visual, auditory, olfactory and gustatory) no conclusive pattern of results emerged. The greatest overlap between the mask and data presented in this thesis occurred with disgusting auditory and olfactory stimuli. There was minimal overlap between the mask and visual and gustatory data presented in this thesis.

7.4 Discussion

There was no overlap between the mask and brain activation in response to facial expressions of disgust (chapter 3) or disgusting tastes (chapter 6, group 2). This is due to the lack of activation in the areas covered by the mask in those two experiments. The paucity of insula activation in response to facial expressions of disgust and disgusting gustatory stimuli has been discussed in chapter 3 and chapter 6, respectively. However, results from experiments in which activation in the insula, putamen, superior temporal gyrus or primary visual cortex were demonstrated (non-verbal vocal expressions of

disgust, chapter 3; facial expressions of disgust, chapter 4; disgusting odours, chapter 5; disgusting tastes, chapter 6) did have areas of activation in common with the overlap mask. These common areas differed between the different sensory modalities, and no clear picture emerged. The insula may, therefore, be involved in the perception of disgusting stimuli regardless of sensory modality of stimulus presentation, as has previously been suggested (Calder et al., 2001). However, since the mask was created solely from visual stimuli, it was not representative of the insula response to stimuli presented in different sensory modalities, and therefore no consistent pattern of overlap emerges. On the other hand, the insula cortex may be involved in the response to visual displays of disgust, including facial expressions (Phillips et al., 1997, 1998b, 1999; Sprengelmeyer et al., 1998; Schienle et al., 2002; Anderson et al., 2003a) and disgusting scenes (Phillips et al., 2000), but not specifically involved in the response to disgusting stimuli presented in other sensory modalities. However, this seems unlikely as insula activation was also demonstrated in response to some auditory, olfactory and gustatory stimuli of disgust in this thesis, though this has not always been consistent.

It is difficult to distinguish between those two possibilities on the basis of the evidence so far. There is evidence that the insula is involved in the perception of disgust in the visual modality (Phillips et al., 1997, 1998b, 1999; Sprengelmeyer et al., 1998; Calder et al., 2000; Schienle et al., 2002; Anderson et al., 2003a), although not all studies have found activation of the anterior insula in response to facial expressions of disgust (Gorno-Tempini et al., 2001). This has been reflected in this thesis. Anterior insula activation was shown in response to facial expressions of disgust in one study (section 4.5) but not in another study (chapter 3).

Few studies have investigated the response of the insula to disgusting stimuli presented in other sensory modalities. Phillips et al. (1998b) failed to find activation of the anterior insula in response to non-verbal vocal stimuli of disgust, but using the same paradigm activation of the anterior insula was observed in response to auditory stimuli of disgust in this thesis (chapter 3). A patient with a lesion of the left insula, putamen and globus pallidus on the other hand was found to be impaired in the recognition of both facial expressions of disgust and auditory stimuli of disgust, and also in the subjective experience of disgust (Calder et al., 2000).

In the olfactory and gustatory modalities there have not been any studies specifically examining neural responses to disgusting stimuli. The results from this thesis have been inconclusive in these sensory modalities. There was increased right anterior insula activation in response to disgusting olfactory stimuli compared with pleasant or unpleasant odours (chapter 5), and there was some left insula activation in response to disgusting tastes in group 2 and left putamen activation in response to disgusting tastes in group 1 (chapter 6).

Other theories suggest that the insula is involved in the monitoring of the internal milieu (Damasio 1994, 1999). This has been supported by a PET study investigating the neural correlates of feeling self-generated emotions (Damasio et al., 2000), in which insula activation was reported in response to all four emotions examined (anger, fear, happiness and sadness). It is possible that this was also occurring in the experiments performed in this thesis. Facial expressions of emotions have indeed been shown to induce a similar category of emotion in the observer (Wild et al., 2001), and both odours and tastes evoke emotional responses (Darwin 1872/1998; Alaoui-Ismaili et al., 1997; Vernet-Maury et al., 1999). This may be an explanation for the insula activation observed in the majority of the experiments.

Future case studies of patients with insula lesions and neuroimaging studies addressing the perception of disgusting stimuli in several sensory modalities will be needed to clarify whether the insula is involved in the perception of disgusting stimuli *per se*, or if it is indeed involved in the mapping and regulation of the internal state and therefore activated by emotion-inducing stimuli.

Chapter 8

General Discussion

In this chapter, the results of the experiments described in this thesis will be reviewed and the implications of the findings for understanding the role of the human insular cortex and other brain regions in the processing of emotions, and especially of disgust, will be discussed.

8.1 Distinct or overlapping neural networks for the processing of different emotions?

The importance of emotions for the guidance of behaviour ensuring survival and adaptation is increasingly being recognized by scientists (Damasio, 1994; Ekman & Davidson, 1994). Consequently, scientists have become increasingly interested in the neural basis of emotions. As described in section 1.2, there are a variety of theories of emotion, which influence the way in which the neural basis of emotions is being investigated: relating emotions to approach and withdrawal or reward and punishment (Rolls, 1999), separating them into specific basic emotions (Ekman, 1992b), or by focussing on different aspects comprising emotion perception (Phillips, 2003). There is evidence that both distinct and overlapping networks are involved in the perception of different emotions, regardless of which approach is followed.

Lesion studies first reported a differential effect of left or right frontal lobe lesions on the behaviour of patients, with left-sided frontal lobe lesions leading to depressive symptoms (Gainotti, 1972). This dichotomy has been supported by electrophysiological recordings, which showed that negative induced affect increased electrical activity in the right prefrontal and temporal lobes, whereas positive induced affect increased brain electrical activity in the left prefrontal and temporal lobes (Davidson, 2002). This finding has been replicated in a PET study (Sutton, 1997). Other groups have found a differentiation between lateral and medial prefrontal cortex with regard to emotion. Northoff et al. (2000) report increased activation in the ventromedial prefrontal cortex in response to negative scenes and in the ventrolateral prefrontal cortex in response to positive scenes. This is in disagreement with a study reporting ventrolateral prefrontal

cortex activation in response to abstract punishment, but ventromedial prefrontal cortex in response to reward (O'Doherty et al., 2001a). Yet another proposal is that internally-generated states and emotions lead to increased blood flow in the ventromedial prefrontal cortex, whereas externally-generated emotions cause an increase in blood flow to the ventrolateral prefrontal cortex (Lane et al., 1998; Yamasaki et al., 2002).

The brain systems involved in the processing of different aspects of emotion perception, such as the identification of an emotional stimulus, the production of a corresponding emotional state, and the regulation of this emotional state, have been described in section 1.3 and have been reviewed by Phillips et al. (2003).

Several lesion and functional neuroimaging studies have been based on Ekman's theory of basic emotions (Ekman, 1992b, a) and have investigated the neural basis of individual basic emotions (anger, disgust, fear, happiness, sadness and surprise). Some of these data have already been described in chapter 1 and in section 3.1. A recent case study (Adolphs et al., 2003) reported a patient with extensive bilateral lesions including the amygdalae, hippocampi, perirhinal, entorhinal and parahippocampal cortices, temporal cortical areas including BA 38, 20 and 21, the basal forebrain nuclei and the anterior insula. Parts of the anterior cingulate cortex and the ventromedial prefrontal cortices were damaged as well. The brain regions which were spared include the posterior inferotemporal cortex, occipiti-parietal cortices, and dorsal and lateral frontal cortices. The patient was impaired at recognizing emotions from static pictures, but could correctly identify emotions from dynamic displays of facial expressions of emotion and from stories describing emotional situations (Adolphs et al., 2003), with the exception of disgust, which was never identified correctly. This study supports the view that the perception of emotions depends on an overlapping network of brain regions, with some emotions being processed in specific brain areas, for example the insula being involved in the perception of disgust to a significantly greater extent than the perception of any other basic emotions. The above paper (Adolphs et al., 2003) also demonstrates that the type of stimulus being used in an experiment can influence the results. The patient tended to label all facial expressions either as happy or as sad, a distinction based on valence. This could be interpreted as emotions being categorized both at superordinate and subordinate levels, with superordinate categories of 'happy' and 'unhappy', and the different basic emotions being at subordinate level. Another

possible explanation for the lack of distinction between emotions beyond happiness and sadness could be that the patient is unable to retrieve knowledge regarding arousal, but is able to retrieve knowledge regarding valence of the emotions. This explanation is in accordance with the suggestion that different emotions can be described in terms of valence and arousal (Lang et al., 1990, 1993).

A recent comprehensive review of neuroimaging studies of emotion (Phan et al., 2002) also supports the view that separate brain regions are involved in different aspects of emotion perception. No single brain region was activated by all emotional tasks, although the medial prefrontal cortex was associated with emotion processing per se, regardless of specific emotion or induction method. In this thesis the medial prefrontal cortex was activated only in response to vocal expressions of anger and fear. The ventromedial prefrontal cortex is involved in decision-making and has previously been linked to emotional tasks that have a large cognitive component (Bechara et al., 1999, 2000; Nakamura et al., 1999; Narumoto et al., 2000). It is possible the lack of activation in the medial prefrontal cortex in most experiments in this thesis is due to the simplicity of the tasks (passive perception or gender discrimination).

Another brain region implicated in the perception of emotion is the anterior cingulate gyrus. It has been proposed that the anterior cingulate is evolutionarily advanced, a specialisation of neocortex rather than a more primitive stage of cortical evolution as it contains a class of spindle-shaped neurons which are only found in humans and great apes (Allman et al., 2001). The evidence from a wide range of studies, including electrophysiological, lesion, and functional neuroimaging studies, suggests a central role for the anterior cingulate gyrus in emotion regulation, motivation, problem solving, error-recognition, goal-directed behaviours and adaptive responses, which are all central to intelligent behaviour (Allman et al., 2001). The anterior cingulate gyrus can be divided into a more rostral and ventral section, thought to be involved in affect, and a more caudal and dorsal section, thought to be involved in cognition (Bush et al., 2000; Devinsky et al., 1995) (also see chapter 3). There is also a posterior part of the cingulate gyrus, which has been implicated in evaluative functions (Vogt et al., 1992) and in processing emotionally salient information (Maddock, 1999). In this thesis activation in the affective division of the anterior cingulate cortex was observed in response to vocal expressions of all negative emotions, facial expressions of disgust, and pleasant tastes.

Activation in the cognitive division of the anterior cingulate was observed in response to vocal expressions of negative emotions except fear, facial expressions of disgust, and disgusting odours. This is not in concordance with a functional division of the anterior cingulate cortex into an affective and a cognitive part. However, since there was a cognitive component at least to the visual and auditory experiments, this could explain the activation in the more cognitive division of the anterior cingulate gyrus. The posterior cingulate gyrus or the retrosplenial cortex was activated in response to vocal expressions of anger and fear, and in response to facial expressions of fear. If this brain area were involved in the processing of emotional stimuli per se, one would expect to see more consistent activation in this area across experiments. In all sensory modalities there was activation of the posterior cingulate gyrus or retrosplenial cortex in at least some experiments in response to the neutral condition. It is uncertain whether this really signifies activation in response to neutral or rather a decrease in blood flow to this area during the emotional condition. These results do not help to elucidate the controversial role (Maddock, 2000; Vogt et al., 2000) of the posterior cingulate or retrosplenial cortex in the processing of emotionally-salient stimuli.

So far brain regions involved in the processing of general emotional processes have been described, and I will now focus on some brain areas which have been associated with specific emotions. The importance of the amygdala for emotional processes has been established in both animals and humans (Aggleton, 1992; LeDoux, 1998). There is a wealth of literature regarding amygdala function, which has been reviewed in detail elsewhere (Buechel & Dolan, 2000; Calder et al., 2001; Davidson, 2002; LeDoux, 2000; Whalen, 1998), I will therefore keep this section brief. The amygdala has been most strongly associated with the perception of fear (Calder et al., 2001). However, functional neuroimaging studies have also observed amygdala activation in response to aversive pictures (Irwin et al., 1996), sad (Blair et al., 1999) and happy (Breiter et al., 1996) faces, positive and negative verbal stimuli (Hamann & Mao, 2002), olfactory (Zald & Pardo, 1997) and gustatory (O'Doherty et al., 2001b; Zald et al., 1998) stimuli, and during aversive conditioning (Buechel et al., 1998; LaBar et al., 1998), to list but a few. These results have been interpreted as suggesting that the amygdala responds to emotional importance or salience regardless of valence (Phan et al., 2002), plays a role in learning new stimulus-threat contingencies and expression of cue-specific fear (Davidson, 2002), is involved in the modulation of vigilance and attention to

emotionally salient information (Phillips, 2003), or acts as a kind of ambiguity detector (Whalen, 1998). In this thesis amygdala activation was observed only in response to vocal expressions of anger and facial expressions of fear, both signals of threat. However, if the amygdala was responsible for learning new stimulus-threat contingencies one would expect to see activation in response to more types of stimuli, as threat is also signalled indirectly through fearful vocal stimuli and directly through angry faces, and also through aversive odours and tastes. For the same reason it seems unlikely that the amygdala responds to emotional importance, as this would predict activation in response to most of the experiments performed in this thesis. Perhaps the vocal angry stimuli and the facial fearful stimuli were the most ambiguous used in this thesis, which would support the view of the amygdala as an ambiguity detector. The non-verbal vocal stimuli of anger were the most ambiguous of the vocal stimuli used in chapter 3 (section 3.3.1.2) as only 70.8% were identified correctly, which is the lowest percentage correct for the auditory stimuli. However, the lowest percentage of correctly identified facial expressions of emotion was also anger (69.5%) followed by sadness (74.3%). This does not support the view that amygdala activation is related to the ambiguity of stimuli. Generally, the sparsity of amygdala activation in this thesis has to be interpreted with caution, as perfect coverage of the inferior temporal lobe during the data acquisition could not be guaranteed (see discussions in chapters 5 and 6, and section 8.4).

The subcallosal cingulate has been associated with the induction of sadness (Phan et al., 2002). No activation in this area was observed in response to facial and vocal expressions of sadness in this thesis (section 3.3.2). Although it has been shown that facial expressions of emotion induce the same emotion in the viewer (Wild et al., 2001) this is not a very powerful method of emotion induction, and the same has not yet been shown for vocal expressions of emotions. As the focus of this thesis is on emotion identification rather than mood induction, the stimuli were not specifically designed to induce sadness, and this could explain the lack of subcallosal cingulate activation.

The suggested role of the anterior insula and basal ganglia in disgust will be discussed in detail in section 8.2.

In summary, there is a large network of brain regions involved in the processing of emotions, including the ventromedial cortex, the amygdala, anterior cingulate gyrus, the insula and the ventral striatum. Lesion studies and functional neuroimaging studies have shown that some parts of this network are involved in certain parts of emotion perception but not in others, and that a single basic emotion can be impaired by certain lesions, sparing the perception and experience of other emotions (for example see (Calder et al., 2000)). It has also been shown that different neural patterns underlie not just the perception, but also the feeling of different emotions (Damasio et al., 2000). In conclusion, different emotions are processed both by overlapping networks and by distinct areas of the brain.

8.2 The anterior insula and disgust

As discussed in previous chapters, there is evidence in the literature that the insula, in particular the anterior insula, is involved in the response to disgusting stimuli. There is some uncertainty whether this is only the case for visual stimuli of disgust, or also for disgusting stimuli presented in other sensory modalities (Calder et al., 2001). Evidence from the experimental chapters of this thesis is contradictory, too. Activation in the anterior insula was observed in response to facial expressions of disgust in some experiments (chapter 4), but not in others (chapter 3). Unlike a previous fMRI study of brain activation in response to auditory non-verbal vocal stimuli of disgust (Phillips et al., 1998), which did not observe activation of the anterior insula, an increase in blood oxygenation in the bilateral anterior insula in response to auditory stimuli of disgust was seen in this thesis (chapter 3). There was also activation of the anterior insula in response to olfactory stimuli of disgust (chapter 5), and to some extent in response to disgusting gustatory stimuli (chapter 6). However, the insula was also activated in response to pleasant tastes.

Lesion studies have indicated that the integrity of somatosensory cortices is critical for the recognition of emotion in others (Adolphs et al., 2000). It has been suggested that the recognition of emotions relies upon an internal reconstruction of what the emotion would feel like via simulation of its associated body state (Adolphs et al., 2000). The close association between recognising an emotion and feeling it has also been shown in a case study of a patient who is unable to feel or identify disgust (Adolphs et al., 2003).

It has been proposed that the insula, especially the anterior insula, is involved in the enteroceptive representation of the body's condition, and that specifically the right anterior insula is integral to mentally generating the image of one's physical state that underlies emotions, a limbic sensory substrate involved in the evaluation that invests internal feelings with emotional significance (Damasio, 1994). This view has been supported by a functional neuroimaging study demonstrating anterior insula activation both during observation and imitation of a mixture of emotions (Carr et al., 2003). However, the prominent role the anterior insula plays in the response to disgusting stimuli as compared to other emotional stimuli, has also been well-documented (Calder et al., 2001). The insula, which is, amongst other things, a visceral somatosensory cortex, is a plausible substrate both for the recognition and the experience of disgust, as this emotion is closely related to ingestive behaviour and evokes such a powerful bodily response (Darwin, 1872/1998; Rozin & Fallon, 1987; Rozin et al., 1994).

A study on fear conditioning in humans has also emphasised the role of the anterior insular cortex in autonomic control and representation, and has proposed that the insula may be important in supporting feedback representation of peripheral autonomic arousal that provides input to conscious awareness of emotional states (Critchley et al., 2002). Such an awareness of autonomic change may provide a core component of the 'feeling' of emotions. Another study reported insula activation during first-order representations of bodily states, which was abolished in patients with peripheral autonomic denervation (Critchley et al., 2001). Both studies support the somatic marker hypothesis put forward by Damasio (1994, 1999).

In functional neuroimaging studies insula activation has been demonstrated in response to thermal sensations (Craig et al., 2000), taste (Kinomura et al., 1994), hunger (Tataranni et al., 1999), odours (Savic et al., 2000), visceral stimulation (King et al., 1999), pain (Schnitzler & Ploner, 2000), anxiety (Reiman, 1997), disgust (Calder et al., 2001), and recall-generated sadness (Mayberg et al., 1999) and during the feeling of sadness, happiness, anger and fear (Damasio et al., 2000). In summary, the insular cortex, especially the anterior insula, appears to be involved in enteroception, and is integral to mentally generating the image of one's physical state that underlies emotions, to the evaluation that invests internal feelings with emotional significance.

In chapter 7 it was investigated if there were a number of regions activated in common to facial expressions of disgust for different studies (Phillips et al., 1997, 1998b, 1999). These areas included the anterior insula, but also the putamen, the superior temporal gyrus, and the posterior inferior temporal gyrus. An application of this mask to the results obtained in this thesis might not be a true reflection of brain areas that are involved in the response to disgusting stimuli presented in different modalities, since the mask is exclusively based upon findings of studies examining the neural response to facial expressions of disgust only. In order to address this possibility, a further mask was created using the results from all experiments in this thesis presenting disgusting stimuli, but did not include the mask based on Phillips et al. data. The work therefore reports neural regions activated in common to stimuli presented in visual, auditory, olfactory, and gustatory modalities. This overlap mask was created from the results presented in section 7.3.2, by overlaying the group brain activation maps reported in this thesis. Therefore only areas of activation reported in GBAMs in the individual experiments were included in this analysis. The mask includes all areas where activation from at least two of the data sets overlapped.

Interestingly, the overlap was not in the anterior insula as expected, but in an area of the frontal cortex adjacent to the anterior insula: the ventrolateral prefrontal cortex (figure 8.2, table 8.2). Exclusion of findings from individual experiments in which no insula activation was observed (facial expressions of disgust: section 3.3.2, disgusting tastes: section 6.3.1) did not alter the nature of this mask, it merely increased the ratio of conditions overlapping from 42% to 60% for BA 47 and from 56% to 80% for BA 45.

This result is interesting, as anterior insula activation was demonstrated in response to most disgusting stimuli in this thesis. Based on this and on previous literature, I therefore expected the overlap of the neural activations in response to multimodal disgusting stimuli to include the anterior insula. Due to certain limitations discussed in the individual chapters and in section 8.4, it is not possible to draw any firm conclusions about the specific role of the anterior insula in the perception of disgusting stimuli presented in multiple sensory modalities, rather than a role in emotional representations per se. Future studies as outlined in section 8.5 might throw more light on this issue.

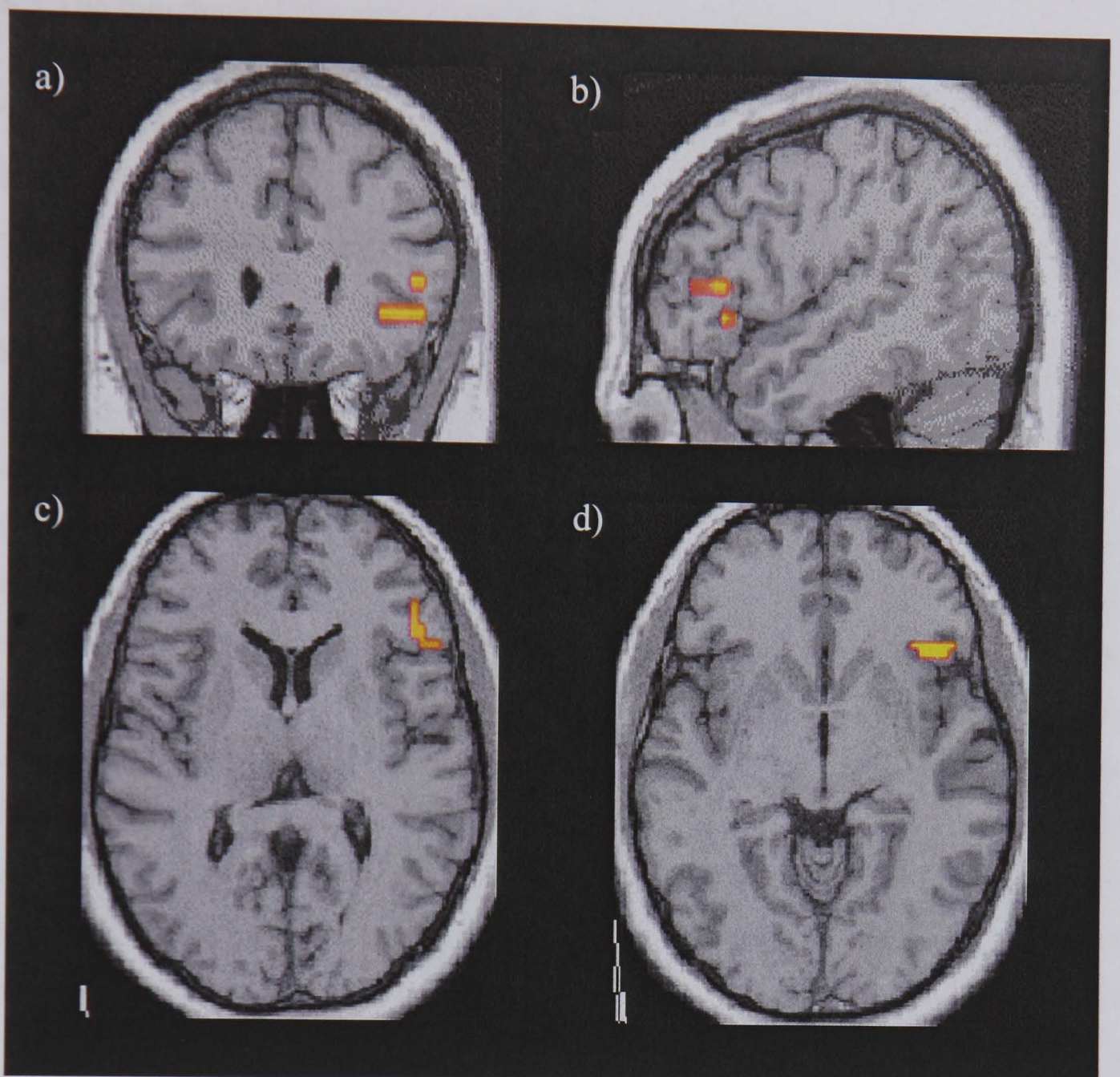


Figure 8.2: Overlap mask.
Overlap of brain areas activated in response to facial expressions of disgust, non-verbal vocal stimuli of disgust, disgusting odours and disgusting tastes. a) Coronal section, y = +26mm, showing the inferior frontal gyrus. b) Sagittal section, x = +46mm, showing activation in the inferior frontal gyrus. c) Axial section, z = +4mm, showing activation in the inferior frontal gyrus (BA 45). d) Axial section, z = -5mm, showing activation in the inferior frontal gyrus (BA 47).

Table 8.2 Overlap mask: main Areas of common activation

Region activated (approximate Brodmann area)	Side	x[a]	y[a]	z[a]	Number of activated voxels	Percent of conditions overlapping
Inferior frontal gyrus (BA 45)	R	43	30	4	8	56%
Inferior frontal gyrus (BA 47)	R	43	26	-7	8	42%

[a] Talairach coordinates refer to the voxel with the maximum FPQ value in each regional cluster.

8.3 The functions of the ventrolateral prefrontal cortex in affect

The ventrolateral prefrontal cortex can be defined as lateral and rostral regions of Brodman Area 47 and part of Brodman Area 45, and lies laterally to the orbitofrontal cortex on the ventral surface of the frontal lobes (Ongur & Price, 2000). Its role in the perception of emotions or generation of emotional states or behaviour is less clear. A neuropathological study examining patients with lesions in the ventral frontal lobe reported socially inappropriate behaviour, subjective changes in emotional experience and impairments in the identification of facial and vocal expressions of emotion (Hornak et al., 1996). Human functional neuroimaging studies have demonstrated increased blood flow to this region in response to a wide variety of tasks, including lip reading (Campbell et al., 2001), viewing positive scenes (Northoff et al., 2000), in response to facial expressions of both happiness and disgust (Gorno-Tempini et al., 2001), different negative emotions (Sprengelmeyer et al., 1998) and anger (Blair et al., 1999; Kesler, 2001), during a gender discrimination task (Lange et al., 2003) and during an emotion expression judgement (Lange et al., 2003; Nakamura et al., 1999; Streit et al., 1999) but not during passive viewing of emotional faces, during the induction of anger (Dougherty et al., 1999) and sadness (Levesque et al., 2003), impulse control and response inhibition (Rahman et al., 2001), empathy (Carr et al., 2003), and embarrassment (Berthoz et al., 2002). These findings suggest a role for the ventrolateral prefrontal cortex in the generation of emotionally-salient associations and in social cognition per se.

In this thesis activation in the ventrolateral prefrontal cortex was observed in response to non-verbal vocal expressions of disgust (chapter 3), facial expressions of disgust (chapter 4), and pleasant, unpleasant and disgusting odours (chapter 5). It is interesting to note that no activation was observed in this area in response to any of the other emotional stimuli presented in chapter 3. Based on previous literature it is unlikely that the ventrolateral prefrontal cortex should play a specific role in the perception of disgust. If, however, the ventrolateral prefrontal cortex plays an important role in the forming of emotionally-salient associations and in social cognition, then it is possible that disgusting stimuli are particularly powerful in activating this region due to their ability to elicit strong emotional reactions and therefore serve as powerful somatic markers (Bechara et al., 2000).

8.4 Limitations of the Experimental Approaches Used in this Thesis

It should be noted that there are some limitations to the methodologies used in this thesis. One of the most prominent limitations of fMRI is that it is correlative in nature. Although one may observe a response that indicates neural activity during a particular cognitive function, it is not possible, using neuroimaging alone, to determine whether that activity is essential for the cognitive function or merely epiphenomenal. Another limitation of fMRI, which has already been mentioned in section 2.1.1, is that the measured BOLD response follows only very indirectly from the underlying neural firing rate. It has been postulated that the haemodynamic response underlying the BOLD signal is driven by energy use, in particular in presynaptic terminals or glia. More recent research has suggested that energy is required predominantly for postsynaptic events and action potentials. Haemodynamic responses may therefore be driven by neurotransmitter-related signalling rather than local cerebral energy requirements (Attwell & Iadecola, 2002). Consequently, the observed activations should be interpreted with the caveat that the neuronal firing rate is not being observed directly, but is instead inferred. Fortunately, there is some evidence to suggest that there is a reasonable concordance between the observed BOLD signal and the underlying neuronal firing rate, at least for some neural regions (Logothetis, 2001; Rees et al., 2000).

Another difficulty with fMRI relates to the localisation of brain activity in the human brain (Brett et al., 2002). Different functional neuroimaging studies have employed a variety of methods of labelling neural regions, including coordinate labels such as the atlas of Talairach and Tournoux (1988), macroanatomical labels relating areas of activation to cortical sulci or gyry, microanatomical labels such as the Brodman Areas, and functional labels as established by Kanwisher et al. (1997). An additional complication in interpreting fMRI data is that applying different statistical analyses to the same data set can show activation in different brain areas (Machulda et al., 2001). The choice of baseline task during the OFF condition has also been shown to influence results (Newman et al., 2001). This, taken together with the variability in brain anatomy between subjects and statistical analysis measures performed to overcome this variability, such as normalisation and smoothing, can make the localisation of

functional MRI data problematic. It is therefore important to pay attention to all these methodological details when trying to interpret fMRI findings.

Accepting these limitations associated with fMRI experiments in general, there are some more specific limitations in the experiments performed in this thesis. Generally, more behavioural measures would have been advantageous throughout the fMRI experiment. In chapters 3 and 4 a sex-differentiation task was employed during the fMRI experiments, which ensured that the subjects attended to the stimuli throughout the experiments. However, future experiments investigating emotion perception could benefit from monitoring physiological variables such as heart rate or skin conductance rate in order to determine whether subjects merely identified emotion or experienced an emotional response. Similarly, EMG measures could be introduced to determine the extent to which subjects mimic the facial expression with which they are presented. In this thesis no behavioural measures were employed in the experiments presented in chapters 5 and 6. This was because performance of certain cognitive tasks has been shown to influence the pattern of brain activation observed (Royet et al., 2001). However, the introduction of a behavioural task might have increased the attention subjects paid to the stimuli. Employing physiological measures such as breathing rate, heart rate or skin conductance responses may have helped to determine the nature of emotional responses in subjects during each study (Vernet-Maury et al., 1999). The objective of all experiments was, however, to examine neural correlates of identification of emotional stimuli rather than emotional experience, although these two components are often difficult to distinguish (Wild et al., 2001). Given the subjective variability in odour ratings, another way to gain insight into the way stimuli were perceived would have been to ask subjects to rate the pleasantness, irritability (in the case of odours), intensity and familiarity of stimuli immediately post-scan using a visual-analogue scale, as has been employed in PET experiments investigating the neural correlates of olfaction (Savic et al., 2002).

Another limitation was the nature of the acquisition sequences used for data collection, as discussed in chapters 5 and 6. Nevertheless, the method of data acquisition was chosen in order to allow comparison with previous experiments, as shown in chapter 7, and to allow the analysis described in section 8.2. Neither of these would have been

possible if the data acquisition had been optimised specifically for orbitofrontal and inferior temporal lobe coverage in chapters 5 and 6.

8.5 Future Directions

Much remains to be elucidated regarding the role of the anterior insula in the perception of disgust across sensory modalities. In this thesis, some evidence was presented that supports a role for the anterior insula in the perception of disgusting stimuli regardless of the sensory modality of stimulus presentation. However, not all results support this hypothesis. There have been some explanations as to the absence of insula activation in response to all the disgusting stimuli presented during the experiments, including an effect of the order and context of stimulus presentation (chapter 4). The absence of insula activation in response to some gustatory stimuli, especially disgusting tastes, could be caused by "pure" tastes not having sufficient power to convey disgust, as pure tastes are limited to sweet, sour, bitter, salty and umami. Flavours are more likely to be encountered. Indeed, Darwin (1872/1998) defined disgust as 'something offensive to the taste', and mentioned examples of food. These are likely to contain a certain element of odour and also somatosensory properties, i.e. flavours rather than pure tastes. They are then more likely to induce disgust.

Future experiments could therefore investigate the components of a flavour, which render it disgusting, and whether it is necessary for a taste to be associated with an odour in order for it to be perceived as disgusting. Somatosensory properties of a stimulus also contribute to its flavour, and should therefore be taken into account when designing an experiment investigating disgusting odours, tastes and flavours. This could be investigated in healthy volunteers, but also in patients with olfactory perceptual abnormalities, such as anosmia, which can be caused by accidents involving head trauma, leading to ablation of the olfactory nerve at the level of the cribriform plate, and in subjects with acute rhinitis. Both patient groups should have a sufficiently diminished or non-existent sense of smell. It should be possible to present these subjects with flavours rated as disgusting by normal volunteers, and determine whether those flavours are rated as disgusting in the absence of an odour. Neural correlates of the response to these stimuli, both when the odorous component can be perceived and when this is not possible, could then be examined with fMRI. All stimuli, odours, tastes and flavours,

could be delivered orally (Cerf-Ducastel & Murphy, 2001). This would ensure comparable somatosensory stimulation across all experiments, as mentioned in chapter 6.

In order to investigate the involvement of the anterior insula in the processing of disgusting stimuli regardless of sensory modality of stimulus presentation one could perform an experiment presenting facial and vocal expressions of disgust, and disgusting odours and tastes or flavours to one subject group. This could be done in a block-design, similar to the experiments described in this thesis, but instead of dedicating a whole experiment to a sensory modality one could focus on disgust only and present stimuli from all sensory modalities in one study. This would allow direct comparison of areas of brain activation in response to disgusting stimuli presented in different sensory modalities in the same subject group, and should therefore clarify the question whether the anterior insula is involved in the perception of disgust per se, regardless of the sensory modality of stimulus presentation.

8.6 Conclusion

Disgust is an important emotion that protects us from potentially harmful stimuli in the environment. In this thesis, using functional magnetic resonance imaging, anterior insula activation has been demonstrated for the first time in response to disgusting stimuli presented in sensory modalities other than the visual modality, namely in the auditory and olfactory modalities. It has also been shown that other neural regions such as the ventrolateral prefrontal cortex play an important role in the perception of emotions, possibly even a role in the perception of disgust, specifically. In this thesis, sophisticated means of presenting olfactory and gustatory stimuli to subjects in an fMRI environment have been developed and successfully applied. Future studies using increasingly sophisticated measures of subjective responses to emotional stimuli will be able to determine the exact nature of the roles of anterior insula and other neural regions in response to disgusting stimuli presented in different sensory modalities.

"*The advantage of the emotions is that they lead us astray, and the advantage of science is that it is not emotional.*" Oscar Wilde, The Picture of Dorian Gray, 1891.

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Appendix

Publications arising from the work in this thesis

Heining, M., Young, A.W. Ioannou, G., Andrew, C.M., Brammer, M.J., Gray, J.A., Phillips, M.L (2003) Disgusting smells activate human anterior insula and ventral striatum. Annals of the New York Academy of Sciences, Vol. 1000, 380-384.

Phillips, M.L. and **Heining, M.** (2002) Disgust and the self. In: Disorders of Body Image. (Eds: D.J. Castle, K. Phillips) Judy Wrightson, Wrightson Biomedical Publishing.

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Disgusting Smells Activate Human Anterior Insula and Ventral Striatum

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KEYWORDS: **Huntington's disease; disgusting smells; anterior insula; ventral striatum**

INTRODUCTION

Patients with Huntington's disease, who initially develop lesions in the striatum,[1,2] and a patient with a lesion of the left insula and striatum[3] are impaired in the perception of facial expressions of disgust. Thus these brain structures may be important for the perception of disgust. Several neuroimaging studies have demonstrated activation of the anterior insula and the ventral striatum in response to facial expressions of disgust, supporting this hypothesis.[4] The anterior insula is also involved in gustation and olfaction.[5] Thus it is unclear whether this region is concerned with all smells or whether it includes a region more specifically involved in the analysis of disgusting tastes or odors. To examine this issue we used functional magnetic resonance imaging (fMRI) to determine brain activity during the presentation of (1) disgusting, (2) pleasant, and (3) unpleasant (not disgusting) odors, each compared to fresh air.

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METHODS

Two groups of eight healthy volunteers (male, right-handed, nonsmoking) each participated in two 5-min experiments during which they were exposed to disgusting (D) odors and, in addition, to either pleasant (P; group 1) or unpleasant but not disgusting (U; group 2) odors. Fresh air served as a neutral control stimulus. All participants performed normally on the UPSIT[6] and gave informed written consent. Subjects rated the stimuli used during fMRI several days before their arranged scan time to verify appropriate hedonic responses to D, P, and U stimuli. The stimuli used were banana (P), vanilla (P), AR300 (acrid rubbish) (D), SK200 (animal feces) (D), CV900 (cat urine) (U), IBQ (musty) (U) (all supplied by Caravansons, Ltd.). During fMRI the 5-min experiments each consisted of blocks of 30-s ON (odors) and 30-s OFF (fresh air). Two different odors of the same category (D, P, or U) were alternated in each ON phase to prevent habituation. Odors were delivered to a facemask at 1 L per min and extracted by vacuum pump to avoid mingling of odors in the facemask. Subjects kept their eyes closed and breathed normally.

Image Acquisition

In each of 14 near-axial planes (7 mm thick, 0.7-mm gap, in-plane resolution 3 mm) T2*-weighted MR images (TE = 40 ms, TR = 3000 ms, theta = 90°, 100 images/slice) depicting BOLD[7] contrast were acquired over 5 min per experiment on a GE Signa 1.5T Neurovascular system. In the same session, a 43-slice, high-resolution inversion recovery echoplanar image of the whole brain was acquired in the AC-PC plane.

Image Analysis

The data were analyzed using Generic Brain Activation Mapping.[8,9] As we wished to compare directly the intensity of activation in response to D, P, and U odors we chose several clusters of activation within the bilateral insula and ventral striatum. The power of functional response was averaged over each cluster. Statistical comparisons of intensity of activation in each cluster were made for the two experimental conditions (D vs. P and D vs. U) by matched-pairs *t*-tests.

RESULTS

Generic brain activation was demonstrated in the left anterior insula in response to all odors contrasted with air, but in the right anterior insula only in response to disgusting odors (FIG. 1). In group 2, additional activation was

demonstrated within the right ventral striatum in response to disgusting odors. In group 1, the comparison of a measure of the mean intensity of activation within the anterior insula and ventral striatum across conditions revealed a significant difference in response to disgusting compared with pleasant odors in the right anterior insula (x = 42, y = 20, z = -2,) at $P = 0.05$. No difference in activation was found in the left anterior insula (x = –31, y =

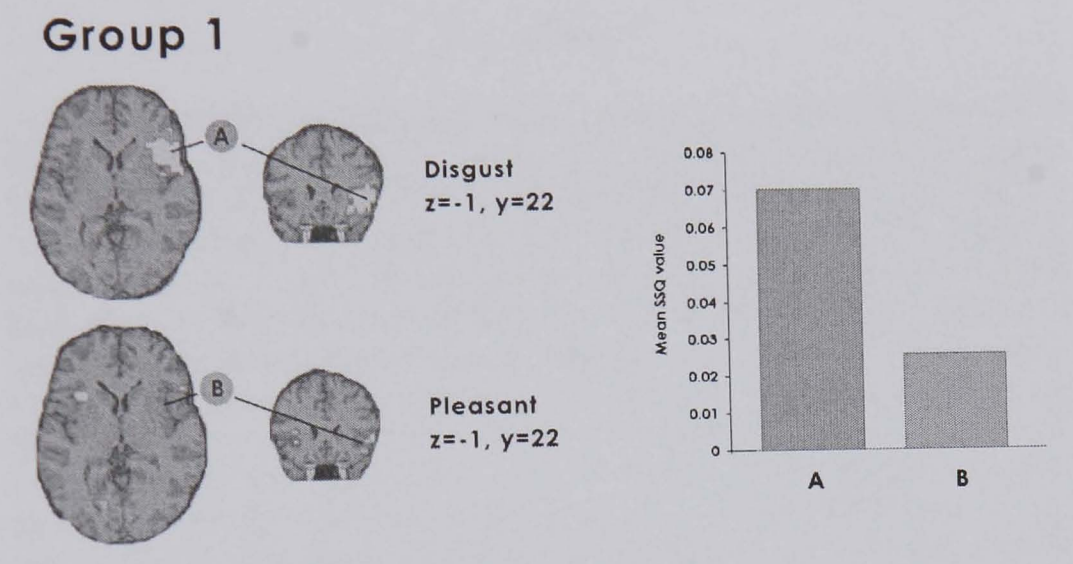

FIGURE 1. Major clusters of generic brain activation in response to disgusting and pleasant (Group 1), and disgusting and unpleasant but not disgusting (Group 2) odors compared with air are demonstrated in axial and coronal brain slices. In group 1, the magnitude of functional response in a cluster within the right anterior insula (x = 42, y = 20, z = –2) was significantly greater ($P = 0.05$) in response to disgusting odors (cluster A) than to pleasant odors (cluster B). In group 2, the magnitude of functional response in a cluster within the right ventral striatum (x = 22, y = 10, z = –7) was significantly greater ($P = 0.01$) (cluster C) in response to disgusting odors than to unpleasant odors (cluster D).

25, z = 4). In group 2, there was a significant difference in BOLD signal change in response to disgusting compared with unpleasant odors in the right ventral striatum (x = 22, y v 10, z = –7) at $P = 0.01$. No statistically significant difference in activation was found in the (a) right or (b) left anterior insula [(a) x = 35, y = 18, z = –2, (b) x = –36, y = 16, z = 4].

DISCUSSION

Disgust is believed to have evolved to provide protection from the risk of contamination and disease. The characteristic facial expression of disgust involves muscles necessary for the avoidance of ingestion of contaminants.[10,11] A close link between systems specialized to react to harmful odors, whether detected directly by the individual or indirectly by way of conspecific facial expression, is likely to have aided survival. Based on the results of previous studies of neural responses to facial expressions of disgust,[4,12] we hypothesized that the anterior insula might be activated by disgusting odors to a significantly greater extent than by pleasant or unpleasant odors. Our results are consistent with this suggestion, but involve unexpected hemispheric lateralization: we demonstrated a significant increase in *right* anterior insula and ventral striatal activation in response to disgusting odors. Left-sided anterior insula activation was demonstrated in response to all odors regardless of valence, reflecting the role of the insula in olfactory perception *per se*.[13]

Our findings demonstrate a specific role of the anterior insula and ventral striatum in the response to disgusting stimuli presented in the olfactory modality. Given previous findings highlighting the importance of these regions in the response also to visual displays of disgust, we propose that the anterior insula and ventral striatum are key components in a system mediating the response to disgusting stimuli irrespective of sensory modality.

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2

Disgust and the self

Mary L. Phillips and Maike Heining

There is necessarily an emotional response to the self, which may be positive or negative. Of all the basic emotions, disgust is an especially powerful negative emotion, and would appear to be an important part of the negative emotional response to the self, and this is inherent in disorders of body image. The aim of this chapter is to discuss the concept of disgust. It provides an overview of recent research examining the neurobiology of emotion in general, and the neurobiology of disgust in particular, and the relationship between the perception of disgust and the perception of the self.

What are emotions?

What are emotions and why do we have them? Dualist, or 'Feeling', theories proposed by Descartes describe emotions as epiphenomena, or non-functional feelings, separate from the physiological changes or behaviours in response to provoking stimuli. Behaviourist theories define emotions in terms of reinforced patterns of behaviour. Cognitive theories, dating from Aristotle, emphasize the importance of cognitions as causal to emotions, with theorists such as Lyons (1992) describing the appraisal of interpretation of events, which then leads to physiological changes, as central to the formation of an emotion. Ekman (1992) has described emotions as 'having evolved through their adaptive value in dealing with fundamental life-tasks'. He argues that emotions are characterized by several unique features, including a distinctive facial expression, distinctive physiology, presence in other primates, and distinctive antecedent events (Ekman, 1992). Intact perception and experience of emotion would thus appear to be vital, in evolutionary terms, for survival in the social environment.

How many different emotions are there? One theory (Lyons, 1992) argues against separate, basic emotions, but instead suggests that a general level of arousal will be interpreted by the individual in terms of the events and evalu-

ations with which it is associated. Davidson (1992) has proposed a single emotion dimension built upon primitive adaptive responses: approach (positive) through to withdrawal (negative). The other type of theory (Darwin, 1872; Ekman, 1992) argues for the existence of separate, basic emotions, proposing six: sadness, happiness, anger, surprise, fear and disgust. This theory has become more popular in recent years as studies have examined the neurobiological substrates for different emotions (see below).

Disgust

Disgust (literally, 'bad taste') has been defined in terms of a food-related emotion. Darwin (1872) wrote that disgust was '... something offensive to the taste', and later authors described the emotion as 'revulsion at the prospect of (oral) incorporation of an offensive object' (Rozin and Fallon, 1987). The objects of disgust have been identified as waste products of the human and animal body. In addition, the concept of disgust can be expanded to involve violation of body borders at points other than the mouth (Rozin and Fallon, 1987). This concept of core disgust can be further elaborated to include: animal-origin disgust, with the tendency of humans to emphasize the human–animal boundary and avoidance of unnecessary contact with animals; interpersonal contamination, with disgust elicited by physical contact with unpleasant or unknown people; and, finally, the moral or socio-cultural domain of the emotion, with disgust at certain beliefs or behaviours, such as sexual abuse of children, acting as a powerful means of transmitting social values (Rozin and Fallon, 1987). It has also been argued that other complex emotions, such as shame, guilt and embarrassment, are derived from the basic emotion of disgust, with the focus of disgust on the self. It is therefore reasonable to hypothesize that an inappropriate or exaggerated perception of disgust, and the complex emotions derived from disgust, would underlie many of the disorders of self and body image.

The neurobiology of disgust perception

Is there a specific neural substrate for disgust?

Lesion and functional neuroimaging studies have been successful in demonstrating the roles of different brain regions in the neural response to different specific emotions in humans. Many of these studies have employed as stimuli facial expressions from the series of Ekman and Friesen (1976) in which subjects view different identities displaying facial expressions of fear, disgust, anger, sadness, happiness and surprise, in addition to a neutral expression. An assumption, which remains to be challenged successfully, is

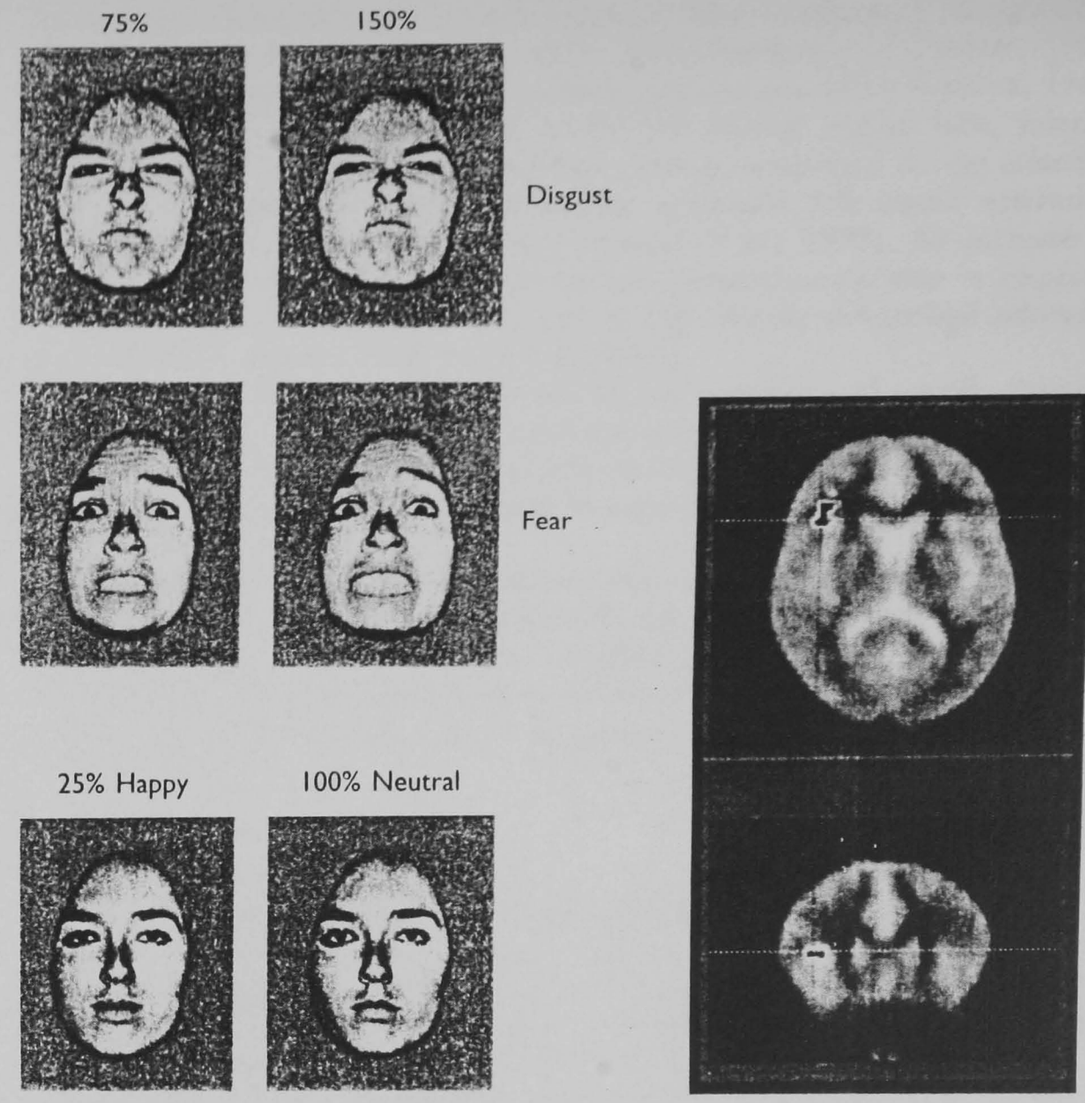

Figure 2.1. (a) Examples of the face stimuli used. Faces from a standard set were computer-transformed to different intensities (75% and 150%). Mildly happy (25%) facial expressions were employed as the neutral baseline stimuli, as it is socially conventional to signal approval and the 100% neutral face stimuli can appear threatening.
(b) Difference image of the activation for perception of facial expressions of 150% (severe) disgust and 75% (mild) disgust. Both transverse and coronal sections show activation of the insula (Talairach co-ordinates 38, 17, 9). Reprinted with permission from *Nature* (Phillips *et al.*, 1997) © 1997 Macmillan Magazines Ltd.

that when viewing a facial expression, subjects also empathize with the emotion displayed. The neural response to a specific facial expression therefore reflects not only the recognition by the subject of that emotion in another, but also the experience, in part, of the emotion.

Using these stimuli, it has been demonstrated that the amygdala is critical to the perception of fear (e.g. Adolphs *et al.*, 1994; Morris *et al.*, 1996). There have been fewer studies examining the nature of the neural response to other specific emotions. One study has, however, demonstrated impaired recognition of disgust from facial expressions in patients with Huntington's disease, in whom lesions in the putamen and other striatal regions are present (Sprengelmeyer *et al.*, 1996). A recent case study examining a patient with lesions to the insula and putamen, reported impaired recognition of disgust from facial expression and from non-verbal vocal stimuli, in addition to significantly lower than normal scores on a disgust experience questionnaire (Calder *et al.*, 2000). These studies indicate the importance of the insula and striatum in the perception of disgust.

Neuroimaging studies have also provided evidence for the role of the insula in particular in disgust perception. In the first neuroimaging study to examine the neural basis of disgust perception with facial expressions of disgust, structures demonstrated to be important for disgust perception included the anterior insula and components of a cortico-striatal-thalamic circuit, particularly the putamen, implicated in appreciation of offensive stimuli in primates (Phillips *et al.*, 1997). The results of this study also indicated the presence of a differential neural response to facial expressions displaying the different specific emotions of fear and disgust: the amygdala was activated in response to facial expressions of fear, but not disgust, and the anterior insula was activated in response to facial expressions of disgust but not fear (Figure 2.1). These findings have been replicated in later studies (Sprengelmeyer *et al.*, 1998).

Is there a similar neural substrate for the perception of disgust in other sensory modalities?

It is important in evolutionary terms that an appropriate behavioural response is made to an emotionally salient stimulus regardless of the sensory modality in which the stimulus is presented. There have been fewer studies examining the neural response to any type of emotional stimulus presented in non-visual modalities. Studies have demonstrated the importance of the amygdala in perception of fearful faces and vocalizations (Scott *et al.*, 1997; Phillips *et al.*, 1998), and further investigation of the neural responses to vocalizations of disgust have suggested involvement of the insula in the perception of vocal expressions of disgust (Heining *et al.*, unpublished data).

Studies of subjects with focal brain lesions, and those employing functional neuroimaging techniques, have also demonstrated a significant overlap between regions important for perception of distinct odours and flavours, and those involved in emotion perception. For example, lesion and functional neuroimaging studies have highlighted the importance of the insula, amygdala, orbitofrontal cortex and temporal lobes in olfactory identification and discrimination (Chitanondh, 1966; Jones-Gotman and Zatorre, 1988; Sobel *et al.*, 1998; Zald and Pardo, 2000; Zatorre and Jones-Gotman, 1991; Zatorre *et al.*, 1992). Activation of the left medial frontal lobe, inferior frontal cortex, and bilateral insulae have been demonstrated during olfaction *per se*; with pleasant odours producing enhanced left insula activation compared with unpleasant odours (Fulbright *et al.*, 1998). An increase in blood flow in left orbitofrontal cortex and bilateral amygdalae in response to perception of unpleasant (although not specifically disgusting) odorants has also been shown (Zald and Pardo, 2000).

Brain areas thought to be important for the perception of specific flavours include the insula, parietal and frontal opercula, and the orbitofrontal cortex (Small *et al.*, 1999). Amygdala and orbitofrontal cortex activation have been demonstrated specifically in response to aversive gustatory stimulation (Zald *et al.*, 1998).

Taken together, these studies indicate that similar brain regions are indeed involved in the perception of emotionally salient stimuli when presented in different sensory modalities: visual, auditory, olfactory and gustatory. In particular, the insula appears to have an important role in the perception of disgust depicted either as a facial expression, or as an emotionally salient odour or flavour.

Pain and disgust

There is evidence from the neuroimaging literature for a similarity between the neural substrates underlying pain and disgust perception. Processing of painful tactile stimuli is thought to occur in many regions of the brain, but has been associated in particular with increased activity in primary and secondary sensory cortices, the anterior cingulate gyrus, the thalamus and the insula. Other types of unpleasant sensory stimulation, including non-painful and painful gastric stimuli, have been shown to activate bilateral central sulcul regions, the insula and the fronto-parietal operculum, with painful gastric stimulation associated with activation in bilateral insulae and the anterior cingulate gyrus (Aziz *et al.*, 1997). These findings indicate that the insula has an important role in perception of pain and disgust. Furthermore, the findings suggest that the experience of disgust as an emotion may be associated with the experience of pain.

Emotion regulation

In the previous section, the roles of the amygdala and insula in the perception and experience of the negative emotions, fear and disgust, respectively, have been discussed. Despite the increasing number of studies employing

functional neuroimaging techniques to examine the neural correlates of emotion perception in healthy volunteers, however, the specific nature of all the components of the neural systems underlying emotion perception, experience and regulation remain unclear. There is some emerging evidence from animal studies, and those employing neuroimaging techniques, for the role of the inferior frontal cortex in the control or inhibition of the experience of negative emotion. Earlier studies have, for example, demonstrated prefrontal activation (as measured by electroencephalogram (EEG) recordings) in subjects with repressive–defensive coping styles (Tomarken and Davidson, 1994); the importance of this structure in the regulation of fear extinction in rats (Morgan *et al.*, 1993); and the impaired ability of patients with prefrontal lesions to make informed decisions about risk-taking behaviours (Bechara *et al.*, 1997). Recent neuroimaging studies have also highlighted the reciprocal roles of the lateral and inferior prefrontal cortex and insula, amongst other regions, in changes in depressed mood. Increases in regional cerebral blood flow (rCBF) in the insula and subgenual cingulate gyrus have been demonstrated to be associated with decreases in rCBF in the right inferior frontal cortex (Brodmann Area (BA) 47) during induction of sadness in healthy volunteers (Mayberg *et al.*, 1999). Inhibition of limbic centres by the prefrontal cortex (and vice versa) has also been inferred from the pattern of rCBF correlations in several human functional neuroimaging studies (Davidson and Sutton, 1995; Drevets and Raichle, 1998).

Further evidence for the importance of the prefrontal cortex in emotion regulation has come from studies of patients with depersonalization disorder. Depersonalization is an alteration in the perception or experience of the self. The sufferer feels uncomfortably detached from their own senses and surrounding events, as if they were an outside observer (DSM-IV) (American Psychiatric Association, 1994). Such symptoms have been found in 2.4% of the general population (Ross, 1991) and in up to 80% of psychiatric inpatients, with 12% experiencing these as severe and persistent (Brauer *et al.*, 1970). Classical descriptions emphasize reduced, 'numbed', or even absent, emotional reactions, e.g. 'all my emotions are blunted' (Shorvon, 1946), and 'the emotional part of my brain is dead' (Mayer-Gross, 1935).

Functional neuroimaging studies in patients with depersonalization disorder have demonstrated left fronto-temporal activation at rest (Hollander *et al.*, 1992), and increased inferior frontal activation associated with reduced or absent insular activation during the viewing of aversive, and particularly disgust-evoking, scenes (Phillips *et al.*, 2000). These findings suggest a reciprocal relationship between activation of brain regions important for the experience of negative emotions such as disgust; that is, the insula and the inferior frontal cortex.

Taken together, these studies indicate a role for the inferior and lateral prefrontal cortices in the regulation of emotion, with the insula and medial prefrontal cortices, including the ventral anterior cingulate gyrus, associated with the experience of negative emotions, particularly disgust.

The neurobiology of self-perception

There are several higher cognitive processes which may be considered relevant to the perception of self (see Keenan *et al.*, 2000). These include the ability to recognize as belonging to the self physical attributes, including, for example, one's own face, speech and body, and the ability to experience specific emotions in response to familiar and self-related sensory information. Of additional importance are other cognitive processes, including the ability to construct a set of self-related, or autobiographical, memories, and the ability to attribute mental states to others (theory of mind). Of particular relevance to body and self image perception would appear to be the ability to recognize different aspects of the self when presented in different sensory modalities, and to experience an appropriate (or inappropriate) emotional response to these physical components of self. Perception of visual and non-visual components of self may be associated, for example, with the experience of disgust. The neural response to visual and non-visual presentations of self-related information may, therefore, include brain regions important in the response to emotionally salient stimuli.

Studies of split-brain patients (i.e. those having undergone forebrain commissurotomy surgery), and studies employing psychophysical (reaction time), and psychophysiological (skin resistance response and measurements of event-related potentials (ERPs)) techniques, have indicated that self-recognition may be associated with the right prefrontal cortex (see Keenan *et al.*, 2000). Other studies of focal brain lesion patients, and those employing functional imaging techniques, have also associated the right prefrontal cortex with autobiographical memory and the perception of self-referential statements (e.g. Fink *et al.*, 1996). Prefrontal regions have also been implicated in the ability to have intact theory of mind (see Keenan *et al.*, 2000).

A recent functional imaging study has examined the neural correlates of self versus non-self information using morphed images of the face of the self and that of another individual of the same sex, in addition to the processing of self-referential information: words describing personality or non-personality traits (Kircher *et al.*, 2000). During recognition of the subject's own face, activation was demonstrated in limbic areas (hippocampus, insula, anterior cingulate), right middle temporal lobe, left inferior parietal lobe and left prefrontal regions. Left-sided areas, including the insula and inferior frontal gyrus, were also activated when subjects judged whether psychological trait adjectives described themselves (Figure 2.2).

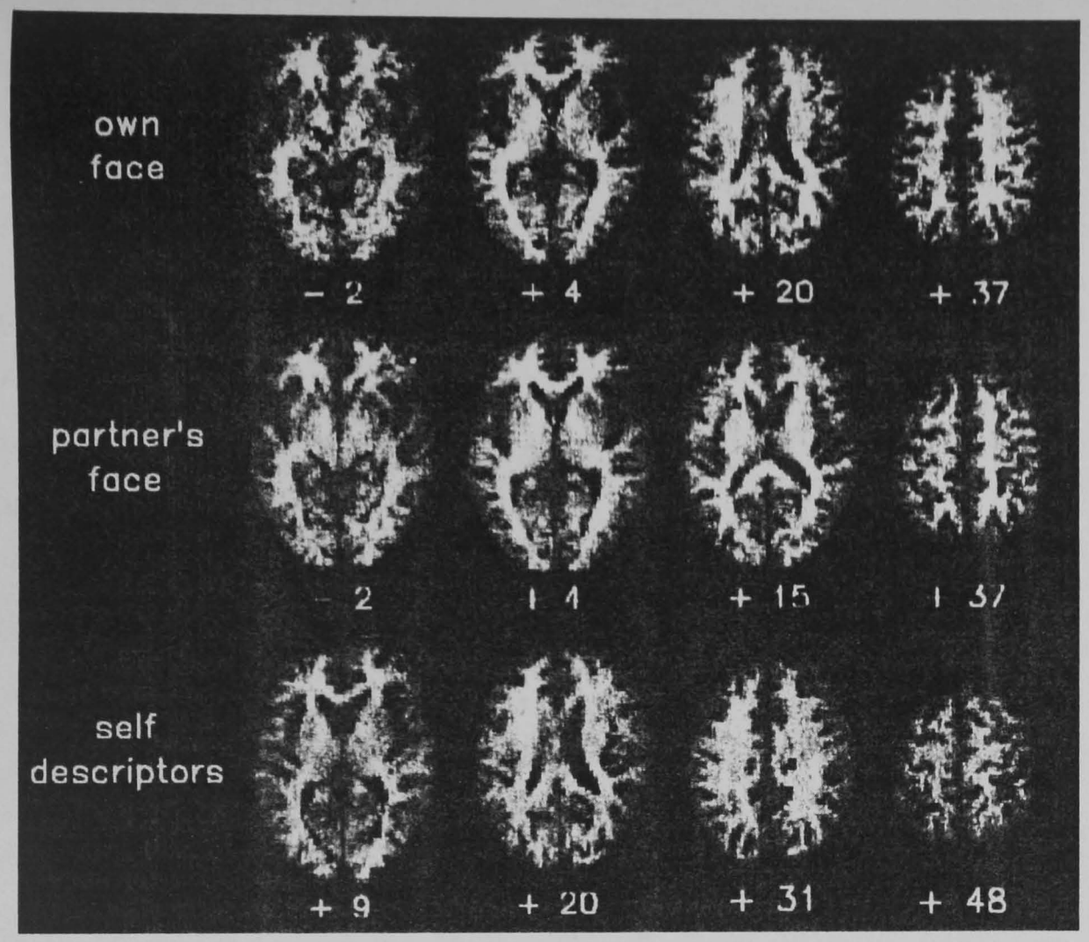

Figure 2.2. Generic brain activation map showing activation during judgement of self-recognition. The numbers below the slices indicate z-axis Talairach co-ordinates. The main regions of activation are the insula, fusiform gyrus, inferior frontal gyrus, subthalamic nucleus and anterior cingulate. Reprinted with permission, from *Cognitive Brain Research* (Kircher *et al.*, 2000), © 2000 Elsevier Science.

The findings from the above study indicate not only the importance of left prefrontal cortical regions for the perception of self-relevant information, including both self-recognition and perception of self-referential words, but that the neural correlates of self-perception include, as predicted above, components of the neural response to emotional stimuli, i.e. the insula and inferior frontal cortex. This may reflect the role of these structures in the perception of emotionally salient information, and particularly negative emotions such as disgust. Self-perception would therefore appear to involve brain regions important in the experience and regulation of emotion.

One possibility, then, is that self-perception involves the co-ordination by prefrontal cortex of activity in structures important for several different cognitive processes, including emotion perception. The influence of prefrontal

cortical activity on other brain regions important for the performance of these different cognitive processes thus may underlie intact perception of the physical self in all sensory modalities, the processing of current, self-related experiences in the context of stored autobiographical memories, the perception of the relationship of the self to others in the social environment, and the normal experience of familiarity and emotion during perception of the physical self and self-related information. Abnormalities of self-perception, accompanied by the experience of inappropriate negative emotion when regarding the self, occurring in some types of body image disorder, may be associated with dysfunctional regulation by the prefrontal cortex of activity in brain regions important for emotion perception.

Conclusion: perception of disgust and perception of self

With the advent of functional neuroimaging techniques it has become possible to examine the neural correlates of sensory and emotion perception. Earlier studies have examined neural substrates for perception of visual stimuli, for example those depicting facial expressions. Later studies have employed techniques to present emotionally salient stimuli in other sensory modalities: auditory, olfactory and gustatory. It is clear from these studies that similar regions, in particular, the insula, amygdala, and inferior and medial frontal cortical regions are implicated in the perception of aversive stimuli presented in all four sensory modalities.

The way we imagine and perceive ourselves is closely related to the perception of an emotionally salient stimulus. Indeed, the perception of self or body image frequently involves a strong emotional component. Findings from recent studies employing a variety of techniques have demonstrated activation of similar areas for the perception of emotionally salient information and for the perception of self. Promising future studies will aim to determine the nature of the specific abnormalities in the neural systems involved in self and emotion perception which underlie body image disorders.

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12

Neural Correlates of Emotion Perception: From Faces to Taste

Mary L. Phillips and Maike Heining

Is there a relationship between the brain regions involved in perception of others' emotions and those important for olfaction and taste? The aim of this chapter is to discuss the nature of emotions, and in particular the studies that have examined brain regions important in the perception of distinct emotions, such as fear and disgust, and then to demonstrate that similar brain regions are involved in the perception of odors and flavors and emotive stimuli presented in other sensory modalities.

1. What Is the Relationship among Olfaction, Taste, and Emotion?

1.1. Emotions

What are emotions, and why do we have them? Dualist, or "feeling," theories proposed by Descartes and, in later years, by James (1890) describe emotions as epiphenomena, or nonfunctional feelings, separate from the physiological changes or behavior seen in response to provoking stimuli. Behaviorist theories, such as that of Skinner (1974), define emotions in terms of reinforced patterns of behavior. Cognitive theories dating from Aristotle emphasize the importance of cognitions as causal to emotions, with theorists such as Lyons (1992) describing the appraisal or interpretation of events, which then leads to physiological changes, as central to the formation of an emotion. Ekman (1992) has described emotions as "having evolved through their adaptive value in dealing with fundamental life-tasks." He argues that emotions are characterized by several unique features, including distinctive facial expressions, distinctive physiology, the presence in other primates, and distinctive antecedent events (Ekman, 1992). Intact perception and experience of emotion would thus appear to be vital, in evolutionary terms, for survival in the social environment.

How many different emotions are there? One theory (Cannon, 1927; Lyons, 1992) argues against separate, basic emotions, and instead suggests that a general level of arousal will be interpreted by an individual in terms of the events and evaluations with which it is associated. Davidson (1992) has proposed a single emotion continuum built on primitive adaptive responses: the range from approach (positive) to withdrawal (negative). The other type of theory (Darwin, 1965; Ekman, Friesen, and Ellsworth, 1982) argues for the existence of separate, basic emotions, proposing six: sadness, happiness, anger, surprise, fear, and disgust. This theory has become more popular in recent years as studies have begun to elucidate the neurobiological substrates for different emotions, as discussed later.

1.2. Olfaction, Taste, Fear, and Disgust

Odors and flavors can be classified as either pleasant or unpleasant, and enjoyed or avoided, respectively. Two specific emotions that therefore would appear to be of relevance to olfaction and taste are fear, important in prompting appropriate avoidance of unpleasant and threatening stimuli in the environment (LeDoux, 1996), and disgust (literally, "bad taste"). Disgust has, indeed, been defined in terms of a food-related emotion. Darwin (1965) wrote that disgust was "something offensive to the taste," and later authors described the emotion as "revulsion at the prospect of (oral) incorporation of an offensive object" (Rozin and Fallon, 1987). Often the objects of disgust have been identified as waste products of human and animal bodies (Angyal, 1941). In addition, the concept of disgust can be expanded to involve causes such as *violation of body borders* at points other than the mouth (Rozin and Fallon, 1987; Rozin, Lowery, and Ebert, 1994). This core concept of disgust has been further elaborated to include *animal-origin disgust*, encompassing the tendency of humans to emphasize the human–animal boundary, avoiding unnecessary contact with animals (Rozin and Fallon, 1987; Rozin et al., 1994). Other causes of disgust include *interpersonal contamination*, with disgust elicited by physical contact with unpleasant or unknown people (Rozin et al., 1994), and, finally, disgust can arise from the *moral or sociocultural domain* (Miller, 1997), with disgust at certain beliefs or behaviors, such as sexual abuse of children, acting as powerful means of transmitting social values (Rozin and Fallon, 1987). It has been argued that other complex emotions, such as shame, guilt, and embarrassment, are derived from the basic emotion of disgust, with the focus of disgust being on the self (Power and Dalgleish, 1997).

Are there similarities in the neural correlates of taste and olfaction and fear and disgust? The nature of the neural substrates underlying perceptions of different

emotions will be reviewed, followed by a discussion of recent studies examining the neural correlates of olfaction and taste.

2. The Neurology of Emotion Perception: Current Issues

Despite increased interest in studies of the neurobiological basis of emotion perception in recent years (LeDoux, 1996), there are several areas in need of further study.

2.1. *Do Different Emotions Have Specific Neural Substrates?*

Lesion studies have implicated both the left hemisphere (Young et al., 1993) and the right hemisphere (Adolphs et al., 1996) in the perception of facial expressions. The valence hypothesis links positive (approach-related) emotions with the left, and negative (withdrawal-related) emotions with the right, frontotemporal brain regions (Davidson, 1995; Davidson and Irwin, 1999), and further evidence for that comes from a recent functional imaging study (Canli et al., 1998). Other authors have provided evidence indicating roles for the orbitofrontal cortex (e.g., Rolls, 1990; Hornak, Rolls, and Wade, 1996; Angrilli et al., 1999) and the retrosplenial cortex (Maddock, 1999) in emotion perception.

Lesion studies and functional neuroimaging studies have also been successful in demonstrating roles for different brain regions in neural responses to different specific emotions in humans. Many of those studies have employed as stimuli the facial expressions from the series of Ekman and Friesen (1976), in which subjects view depictions of faces displaying expressions of fear, disgust, anger, sadness, happiness, and surprise, in addition to a neutral expression. Those facial expressions can be transformed with computer software to depict different intensities of the emotions (Calder et al., 1997). There has been an assumption, which has not yet been refuted, that when viewing a facial expression, subjects empathize with the emotion displayed. The neural response to a specific facial expression therefore reflects not only recognition by the subject of that emotion in another but also the experience, in part, of the emotion.

With the use of such stimuli, it has been demonstrated that the amygdala is critical to perception of fearful facial expressions (Adolphs et al., 1994, 1995; Young et al., 1995; Breiter et al., 1996; Calder et al., 1996; Morris et al., 1996). Adolphs et al. (1994) demonstrated that patients with bilateral amygdala lesions were impaired in their ability to perceive facial expressions of fear, as well as their ability to match facial expressions. In a functional neuroimaging study employing positron-emission tomography (PET), Morris et al. (1996) demonstrated

a positive correlation between the rate of amygdala blood flow and the intensity of fear depicted in a facial expression.

Although there have been fewer studies examining the nature of neural responses to other specific emotions, the anterior insula and components of a cortico-striatal-thalamic circuit implicated in perception of offensive stimuli in primates (Alexander, Crutcher, and DeLong, 1990), but not the amygdala, have been demonstrated to be important in the perception of facial expressions of disgust (Phillips et al., 1997). That study was the first to demonstrate differential neural responses to stimuli depicting different specific emotions.

Other recent studies have employed facial expressions to examine the neural correlates of perception of other basic emotions. Such studies have demonstrated the importance of the medial frontal cortex and the amygdala (Schneider et al., 1997; Blair et al., 1999) for recognition of sad facial expressions, and the orbitofrontal cortex and anterior cingulate gyrus for recognition of angry facial expressions (Blair et al., 1999). Studies examining the neural correlates of happiness have been inconclusive. Several different brain regions have been implicated in the neural response to this emotion, such as widespread decreases in blood flow over the whole brain (George et al., 1995), amygdala activation (Schneider et al., 1997), and activation in bilateral posterior cingulate gyri and medial frontal cortex and the left anterior cingulate gyrus (Phillips et al., 1998a). Such findings suggest that perception of a given positive emotion may not be dependent on activity in a discrete brain region, but instead may be associated with simultaneous activities in several different brain regions. Furthermore, positive emotions such as happiness may be processed in terms of the environmental context in which they exist, so that happiness occurring in the context of fear might be associated with activity in brain regions different from these activated during the processing of happiness occurring in an otherwise neutral context. It is possible, therefore, that happiness may be a more complicated and less "basic" emotion than previously thought. That problem will be the subject of future studies.

Other types of visual stimuli have been employed in studying the neural correlates of emotion perception. Emotive scenes from the International Affective Picture System (IAPS) (Lang, Bradley, and Cuthbert, 1997) have been employed in several studies. The IAPS comprises scenes rated as depicting different intensities of positive (pleasant), negative (aversive), and neutral emotions. When subjects have viewed those scenes, activation has been demonstrated predominantly in bilateral visual cortical regions (e.g., Lane et al., 1997; Lang et al., 1998; Lane, Chua, and Dolan, 1999). Other studies have employed emotive film excerpts, demonstrating in response to those stimuli activation in occipitotemporal

cortex and limbic structures (Reiman et al., 1997) and medial prefrontal cortex (Paradiso et al., 1997). The results of those studies indicate that regions important for visual-object processing are activated to a greater extent by emotionally salient stimuli than by neutral, complex visual stimuli, despite efforts to control for the degree of visual complexity (luminance, color, detail) contained in the two types of stimuli. Some authors have suggested that that may reflect a modulation of activation in visual regions by regions important for emotion perception, such as the amygdala (Morris et al., 1998).

2.2. *Are Specific Neural Substrates for Perception of Different Basic Emotions Supramodal?*

It is important in evolutionary terms that an appropriate behavioral response be made to an emotionally salient stimulus, regardless of the sensory modality in which the stimulus is presented. There have been fewer studies examining neural responses to emotional stimuli presented in nonvisual modalities. One study to investigate perception and evaluation of the emotions projected by spoken words has demonstrated increased blood flow in the frontal cortex and cerebellum (Imaizumi et al., 1997). Impaired perception of fearful and angry vocalizations has been reported in a patient with bilateral amygdala lesions (Scott et al., 1997), and perception of fearful vocalizations has been reported to be associated with interactions between the amygdala and brainstem regions (Morris, Scott, and Dolan, 1999). In another study, amygdala activation to both fearful faces and sounds was reported in the same group of individuals, with the findings also suggesting a possible general role in emotional behavior for the superior temporal gyrus (Phillips et al., 1998b). A recent study (Blood et al., 1999) has shown a correlation between increasing dissonance or consonance of music and activity in areas of the brain involved in emotion processing, including the right parahippocampal gyrus and precuneus, bilateral orbitofrontal cortex, medial subcallosal cingulate gyrus, and right frontal polar regions.

Processing of painful tactile stimuli is thought to occur in many regions of the brain, but has been associated in particular with increased activity in primary and secondary sensory cortices, the anterior cingulate gyrus, the thalamus, and the insula (Casey et al., 1994; Oshiro et al., 1998). Other types of unpleasant sensory stimulation, including nonpainful and painful gastric stimulation, have been shown to activate bilateral central sulcal regions, the insula, and the frontoparietal operculum, with painful gastric stimulation associated with activation in bilateral insulae and the anterior cingulate gyrus (Aziz et al., 1997). Those findings are further support for the roles of limbic, frontal, and sensory cortical regions in the perception of emotionally salient stimuli, but further studies are needed to increase our understanding of the nature of the neural responses to different types of emotional stimuli presented in different sensory modalities.

2.3. *Emotional Learning*

Some authors have examined the neural correlates of the processes of learning and recalling emotionally salient events by asking subjects to view and subsequently remember events with either an emotional or nonemotional significance. Activation in the amygdala has been associated with the ability to remember emotionally salient stimuli and events (Cahill et al., 1996; Phelps and Anderson, 1997; Hamann et al., 1999), but further studies are needed to clarify the nature of the different neural systems underlying the learning and recall of emotional material.

Other studies have employed conditioning paradigms to determine the neural correlates of emotional learning. It has been well established from studies with non-human primates that fear conditioning involves the amygdala (e.g., LeDoux, 1996; Quirk, Armony, and LeDoux, 1997). In some functional imaging studies, human subjects have been allowed to develop a conditioned fear response to a stimulus such as an angry face paired with the nonconditioning stimulus (often a burst of white noise), with subsequent examination of the neural correlates of their perceptions of the conditioning stimulus (Buchel et al., 1998; LaBar et al., 1998). The results indicate that the amygdala is important for fear conditioning. Those and other studies (e.g., Breiter et al., 1996) have also highlighted the phenomenon of habituation of the amygdala response to fearful stimuli over time. Further studies employing more sophisticated methods of analysis, in which the temporal nature of the responses of specific brain regions implicated in the perception of specific emotions (time-series analysis and event-related fMRI paradigms), will help to clarify our understanding of the neural networks involved in emotion conditioning and learning.

3. Neural Correlates of Olfaction and Taste

The neural correlates of perceptions of negative emotions, particularly fear and disgust, have been shown to include the amygdala and insula, in addition to sensory and medial frontal cortices. The neural correlates of perceptions of pleasant and unpleasant odors and flavors might be predicted to include those structures. There have been fewer studies examining the neural correlates of olfaction and taste, and especially few examining neural responses to emotionally salient odors and flavors. In this review, we attempt in particular to compare the neural

correlates of perceptions of emotionally salient odors and flavors with the neural responses to the basic emotions of fear and disgust.

3.1. Neural Correlates of Olfaction

Receptor cells from the olfactory epithelium project across the cribriform plate and innervate the olfactory bulb; the primary projection of the olfactory bulb neurons are to the piriform cortex, olfactory tubercle, anterior olfactory nucleus, amygdala, and entorhinal cortex (Price, 1987). The olfactory system is in fact the only sensory system that bypasses the thalamus and has direct projections to cortical areas. Lesion studies have highlighted the importance of the orbitofrontal cortex and temporal lobes in olfactory identification (Jones-Gotman and Zatorre, 1988) and discrimination tasks (Zatorre and Jones-Gotman, 1991), and the greater importance of the right, rather than left, anterior temporal cortex in olfactory memory (Rausch, Serafetinides, and Crandall, 1977). Furthermore, the role of the amygdala in olfaction is indicated by the demonstration that amygdalotomy is a successful treatment for olfactory hallucinations in patients with seizure disorders (Chitanondh, 1966).

Neuroimaging studies have highlighted the roles of the orbitofrontal cortex (e.g., Zatorre et al., 1992; Sobel et al., 1998) and the right frontal lobe in olfaction, with the latter activated to a greater extent in females than in males (Yousem et al., 1999). The roles of the piriform cortex, orbitofrontal cortex, amygdala, and entorhinal/hippocampal region in olfaction have been emphasized in a recent review of PET and fMRI studies of the human olfactory system (Zald and Pardo, 2000).

With regard to examination of the neural correlates of perceptions of emotionally salient odors, activation of the left medial frontal lobe, inferior frontal cortex, and bilateral insulae has been demonstrated during olfaction per se, with pleasant odors producing increased left insula activation, as compared with unpleasant odors (Fulbright et al., 1998). Another study has reported increased blood flow in the left orbitofrontal cortex and bilateral amygdalae in response to perception of unpleasant (although not specifically disgusting) odorants (Zald and Pardo, 1997). Perceptions of familiar odorants have also been associated with right orbitofrontal cortical activation (Royet et al., 1999). Although such studies have been few, the findings to date indicate that many of the brain regions associated with emotion perception are also involved in perception of olfactory stimuli: the orbitofrontal cortex, amygdala, and insula. Because odors are rarely devoid of emotional salience, it is probable that the involvement of the orbitofrontal cortex, amygdala, and insula demonstrated in the neural response to olfactory stimulation reflects, at least in part, processing of the emotional component of such a stimulus.

3.2. Neural Correlates of Taste Perception

There have been few lesion studies examining taste perception in subjects with lesions in higher cortical areas, rather than the brainstem or midbrain. Some epilepsy patients experience gustatory hallucinations, which can be induced by stimulation of the parietal or rolandic operculum (Hausser-Haw and Bancaud, 1987). According to a comprehensive review of studies investigating neural substrates for taste perception (Small et al., 1999), the insula, parietal and frontal opercula, and orbitofrontal cortex have been demonstrated to play important roles, predominantly within the right hemisphere. Those authors have suggested that orbitofrontal activity may be dependent on the motivational features of specific taste tasks, whereas the right hemisphere predominance for taste perception may be a result of the specialization of the left hemisphere for language. One study has also reported activation of the anterior cingulate gyrus and thalamus, in addition to the areas mentioned earlier (Faurion et al., 1998). Others have emphasized the importance of activation in the insula and perisylvian region and have reported functional lateralization of taste perception related to handedness in the inferior part of the insula (Cerf et al., 1998; Faurion et al., 1999). In one study in which neural responses to aversive gustatory stimulation were examined, amygdala and orbitofrontal cortex activation was demonstrated specifically in response to such stimulation (Zald et al., 1998).

The findings in such studies indicate that there is significant overlap between regions important for perception of distinct flavors and those involved in emotion perception. Flavors, like odors, frequently have emotional significance, and it is probable that they too are processed to a large extent in terms of their emotional component.

3.3. Examination of the Overlap among Neural Responses to Emotional, Olfactory, and Gustatory Stimuli

The results from the studies just discussed indicate that similar brain regions are activated by emotional stimuli presented in the visual modality and by odors and flavors – both pleasant and unpleasant. The potential overlap between brain regions activated by emotional stimuli presented in different sensory modalities, including the olfactory and gustatory modalities, was investigated directly in a recent study (Francis et al., 1999). Neural responses to a pleasant touch (velvet), a pleasant olfactory stimulus (vanilla), and a pleasant gustatory stimulus (glucose) were examined in the same group of four subjects. Similar (medial) regions of the orbitofrontal cortex were activated by all three types of stimuli, particularly right-sided. The pleasant olfactory and gustatory stimuli also activated similar regions of the bilateral anterior insulae. Those authors suggested that their

findings provided evidence of a role for the orbitofrontal cortex in particular in the learning and representation of rewards, and they argued that emotions per se can be defined in terms of states elicited by reward (or punishing) stimuli. The findings of that and other studies therefore highlight the role of the orbitofrontal cortex and insula in emotion processing. Furthermore, odors and flavors appear to activate these regions in particular, suggesting that these stimuli are processed primarily in terms of their emotional salience.

4. Conclusion: From Faces to Olfaction and Taste

With the advent of functional neuroimaging techniques, it has become possible to examine the neural correlates of sensory and emotion perception. The earlier studies concentrated on investigation of neural substrates for perceptions of visual stimuli depicting facial expressions and unpleasant and pleasant scenes. Later studies have employed techniques to present emotionally salient stimuli in other sensory modalities: auditory, tactile, olfactory, and gustatory. It is clear from these studies that similar regions, in particular the insula, amygdala, and primary sensory and orbitofrontal cortical regions, are implicated in the perception of aversive stimuli presented in all five sensory modalities. The orbitofrontal cortex and insula are also activated by several different types of odors and flavors. This suggests that emotion processing and perception of odors and flavors have similar neural bases and that olfactory and gustatory stimuli seem to be processed to a significant extent in terms of their emotional content, even if not presented in an emotional context. The aim of future studies in this area will be to determine the nature of the specific neural systems involved in olfaction, taste, and emotion perceptions by employing analysis techniques to allow examination of the temporal and functional relationships between the different brain regions identified in these processes.

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